Germination thresholds of the Mixed-grass Prairie species as affected by global climate change: A FACE study.

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ABSTRACT

The effects of global climate change on seed germination and plant regeneration have been reported in many species. However, there are no consistent trends in how seed quality and germination are affected by these conditions. Seeds of four native, one invasive, and two pairs of native/invasive species were collected from the USDA-ARS Prairie Heating and CO_2 Enrichment Experimental plots in 2007 to 2009, located in the Mixed-grass Prairie near Cheyenne, WY. Field treatments include ambient (385 ppm, c) and elevated (600 ppm, C) CO_2 concentrations, control temperature (t) and heating (1.5/3.0°C warmer day/night, T), and deep (ct-d) and shallow (ct-s) irrigation. Seed quality was evaluated and germination tests were conducted under alternating temperatures (10/0, 12.5/2.5, 15/5, 20/10, 25/15, 30/20, 35/25°C). Thermal time requirements (θ_{50}) and base temperatures (T_b) for germination were determined using thermal time models. Elevated CO_2 concentrations reduced seed fill and viability, germination and germination rate in *Grindelia squarrosa*. Heating increased seed viability in *Koeleria macrantha* from 56% to 79%. Heating, when combined with elevated CO_2 concentrations, increased germination while CO_2 alone decreased germination by about 14% in *Bouteloua gracilis*. Heating tended to enhance T_b and to reduce θ_{50} in *Bouteloua gracilis*, which may slow the initiation of germination but seeds germinate faster in spring. Elevated CO_2 concentrations tended to increase but CT tended to decrease T_b in *Centaurea diffusa*, but not θ_{50}. Heating tended to increase but all the other treatments tended to reduce T_b in *Lanaria dalmatica*, leading to a possible earlier start of germination. Irrigation treatments tended to show similar trend of effects on seed quality and germination as that in elevated CO_2 concentrations. Species

specific changes in seed quality and germination were observed, which may exert substantial cumulative effects on community composition in the long run. Invasive species may be more competitive under future climatic conditions compared with native species. However, the distribution and abundance of some native species, specifically *Bouteloua gracilis*, may be favored by climate change.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ct	Ambient CO_2, Controlled Temperature, Non-irrigation
cT	Ambient CO_2, Heated Temperature, Non-irrigation
Ct	Elevated CO_2, Controlled Temperature, Non-irrigation
CT	Elevated CO_2, Heated Temperature, Non-irrigation
ct-d	Ambient CO_2, Controlled Temperature, Irrigation – Deep Irrigation
ct-s	Ambient CO_2, Controlled Temperature, Irrigation – Shallow Irrigation
GLM	Generalized Linear Model
IRGA	Infra-Red Gas Analyzer
LSD	Least Significant Difference
NMP	Northern Mixed Prairie
PHACE	Prairie Heating and CO_2 Enrichment
PID	Proportional Integral Derivative
RCBD	Randomized Complete Block Design
TZ	Tetrazolium Chloride
USDA	United States Department of Agriculture
USDA-NRCS	United States Department of Agriculture Natural Resources Conservation Service

Chapter 1 INTRODUCTION

Warming of the earth's climate system is evident from observations of increases in average air and ocean temperatures, widespread melting of snow and ice, and rising sea level (IPCC, 2007b). Increased concentrations of greenhouse gases (GHG) in the atmosphere due to human activities, mainly fossil fuel use and land-use changes, are thought to be the causes of global climate change. Carbon dioxide is the most important anthropogenic greenhouse gas. Since the industrial revolution, global atmospheric concentration of CO_2 has increased from about 280 ppm to 379 ppm in 2005. The concentration of atmospheric CO_2 has exceeded the natural range over the last 650,000 years (IPCC, 2007a). Moreover, global emissions of greenhouse gases will continue to grow over the next few decades. It is predicted to increase by 25 to 90% (CO_2-eq) between 2000 and 2030 (IPCC, 2007a). Increasing atmospheric CO_2 can cause global warming; since 1900, global average temperature has risen about 1.5°C. A warming of about 0.2°C per decade for the next two decades is predicted by a range of emissions scenarios(Nakićenović *et al.*, 2000). Continued increases in GHG, especially CO_2 emissions at or above current rates would cause further warming and induce many changes in the global climate system, thereby potentially affect all aspects and processes of natural ecosystems (Hogg & Hurdle, 1995; Iverson & Prasad, 1998).

Studies on the effects of elevated CO_2 on plants have focused primarily on the vegetative phase of plant growth (Poorter, 1993&, 1998; Poorter & Nagel, 2000; Poorter & Pérez-Soba, 2001). Functional groups of plants that have shown consistent and distinctive vegetative responses to elevated CO_2 concentrations are reflected in photosynthesis and nutrient utilization

(Poorter, 1993; Wand *et al.*, 1999). Physiological changes under high CO_2 concentrations such as reductions in leaf nitrogen concentrations, accumulation of leaf starch, and down regulation of photosynthesis (Stitt & Krapp, 1999; Körner, 2000) may affect the carbon and nitrogen supply available to reproductive organs (Lawlor, 2002) and in turn constrain the magnitude of potential responses to high CO_2 concentrations during this phase of the life cycle. Plant reproduction in response to rising CO_2 has important implications in natural (Körner *et al.*, 1996) and agro-ecosystems (Wittwer, 1995; Bazzaz & Sombroek, 1996; Rosenzweig & Hillel, 1998). Changes in plant reproductive success could alter the composition and hence the functioning of plant communities (Bazzaz & Sombroek, 1996; Grünzweig & Körner, 2001a).

The effect of CO_2 enrichment, increasing temperature and other parental conditions during plant growth and seed production and its influence on seed germination has been reported in many species (Wulff & Alexander, 1985; Garbutt *et al.*, 1990; Andalo *et al.*, 1996). However, there is no consistent trend within and among plant functional groups regarding seed quality and germination as affected by climate change conditions, possibly due to weakness in the understanding of underlying mechanisms. Newton (1991) observed a trend towards higher seed production per plant and greater individual seed weight, but a variable response in seed numbers, among nine pasture and old-field species at elevated compared with ambient CO_2. A meta-analysis on the effect of elevated CO_2 concentrations on eight reproductive traits on 79 crop and wild species shows that CO_2 enrichment resulted in more flowers (+19%), more fruits (+18%), more seeds (+16%), greater individual seed mass (+4%), greater total seed mass (+25%), and lower seed nitrogen concentrations (−14%) (Jablonski *et al.*, 2002). Elevated CO_2 concentrations has positive effects on germination rate, seed production and seed quality of paper birch (*Betula papyrifera*) (Darbah *et al.*, 2007). In a 5-year study in natural nutrient-poor calcareous grassland,

Thürig et al. (2003) found a 42% increase in seed number ($P < 0.01$) of graminoids, a 33% increase ($P = 0.07$) in forbs, and no significant change in legumes (- 38%, n.s.) under elevated CO_2 concentrations. In most species, seeds tended to be heavier (+ 12%, $P < 0.01$), and N-concentration of seeds was significantly reduced in eight out of 13 species. The fraction of germinating seeds did not differ but the time to germination was significantly shortened in elevated CO_2 concentrations (Thürig *et al.*, 2003).

Greater seed viability under recent and artificial warming have been reported in alpine and high-latitude ecosystems (Wookey *et al.*, 1995; Kullman, 2002; Lantz *et al.*, 2009). In semi-arid temperate grassland, heating did not directly enhance seed viability in *Leymus chinensis* (Gao *et al.*, 2012). But it reduced the proportion of light seeds, which might have low viability. The few studies on the impact of climate warming on individual seed mass are mainly from arctic and alpine ecosystems (Wookey *et al.*, 1995; Graae *et al.*, 2008; Milbau *et al.*, 2009). Positive effects have been reported in herbaceous plants in forests (De Frenne *et al.*, 2010), alpine herbaceous species (Sandvik & Eide, 2009), and a native perennial grass (Gao *et al.*, 2012). However, among 15 species studied on a calcareous grassland, seed mass was not significantly affected by warming after 3 years (Hovenden *et al.*, 2007). Parental warming during plant growth and seed development increased the subsequent seed germination in shrub and forb species in subarctic (Graae *et al.*, 2008; Milbau *et al.*, 2009), forest tree and herbaceous species (Thompson & Naeem, 1996; Chidumayo, 2008; De Frenne *et al.*, 2010), and a grass species, *Themeda triandra* (Williams *et al.*, 2007); decreased seed germination percentage in a dwarf shrub species in subarctic (Graae *et al.*, 2008), three savanna woody species in Africa (Chidumayo, 2008), and grass species on the Eurasian grassland (Gao *et al.*, 2012) and

Australian temperate grassland (Williams *et al.*, 2007); but did not affect the heavy weighted seeds of *Leymus chinensis* (Gao *et al.*, 2012) and in 20 species in subarctic (Milbau *et al.*, 2009).

Most previous studies have focused on a single factor of global climate change, meaning either elevated CO_2 concentrations or parental warming but rarely looking at the combination of the two. With Prairie Heating and CO_2 Enrichment (PHACE) facility, we were able to detect the effects of both factors and their combination, which better resemble future climate condition. The all year round 1.5/3.0°C (day/night) elevated temperature was a moderate level of heating compared with that in other studies. Such approach would more accurately mimic the future climate condition and thus help us better understand the response in regeneration of species on the Mixed-grass Prairie and the mechanism behind it.

We propose to use thermal time models to identify temperature thresholds during germination, and to link these thresholds parameters to seed properties under climate change conditions. Thermal time models have been used to predict germination in a variety of species including common lambsquarters (*Chenopodium album* L.), winterfat (*Eurotia lanata* (Pursh) Moq.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), lentil (*Lens culinaris* Medik.), and many grasses (Ellis & Barrett, 1994; Hardegree & Van Vactor, 1999; Wang *et al.*, 2004; Larsen & Bibby, 2005; Hardegree, 2006; Qiu *et al.*, 2006).

The objectives of this research were: 1) to identify the responses in seed fill, seed viability, individual seed mass and germination in selected species from the Mixed-grass Prairie; 2) to identify the shifts in germination thresholds of those species as affected by global climate change; and 3) to identify the response of invasive species and plant invasion under future climatic conditions on the Mixed-grass Prairie.

With those, we hypothesized that: 1) greater percentage of empty seeds are produced under parental elevated CO_2 concentrations environment but lower percentage under parental warming, with no significant change under elevated CO_2 concentrations and heating; 2) parental heating increases while elevated CO_2 concentrations decreases seed viability; 3) both parental heating and CO_2 enrichment increase individual seed mass; 4) seed germination response to heating and/or elevated CO_2 concentrations is species-specific; 5) future climatic conditions benefit invasive species over native speices in regeneration; 6) Reproduction of *Linaria dalmatica* will positively response to global climate change.

Chapter 2 LITERATURE REVIEW

2.1 Global climate change and the Mixed-grass Prairie

2.1.1 Global climate change

Although several assumptions about economic growth, technological advances, and carbon sequestration by biological and geological processes are required to predict future atmospheric CO_2 concentrations, atmospheric CO_2 concentrations has been and will continue to increase at least for the next several decades (Nowak *et al.*, 2004). The global atmospheric concentration of CO_2 has increased from about 280 ppm since the industrial revolution to 379 ppm in 2005 (IPCC, 2007a). It is expected to increase by 25 to 90% (CO_2-eq) between 2000 and 2030 (Nakićenović *et al.*, 2000). Elevated atmospheric CO_2 concentrations can cause global warming, and alter the amount and patterns of precipitation. The average global temperature has risen about 1.5°C since 1900. A warming of about 0.2°C per decade for the next two decades is predicted(Nakićenović *et al.*, 2000). It is projected that the temperatures in June, July, and August in central North America will increase 3 to 9°C by the late 21st century. The best available projection for summer precipitation is that it will stay at a similar amount to that from 1970–1999, or decline 10–30% by the end of the 21st century with a small chance of increase (Wuebbles & Hayhoe, 2004; Christensen *et al.*, 2007). The future climate will be characterized by more frequent growing season droughts and extreme rainfall events (Harper *et al.*, 2005). A change toward lower precipitation-to-evaporation ratios and drier summers, along with greater

temporal variability in precipitation, more droughts, shorter winters, and longer growing seasons are therefore the most probable scenarios.

As an essential resource for photosynthesis, enriched atmospheric CO_2 concentrations can cause direct physiological changes to plants, directly affecting plant growth (Newton, 1991). Plant production is also affected by soil conditions and nutrient availability. Elevated atmospheric CO_2 concentrations itself can improve soil water relations by reducing transpiration through partial stomatal closure (Field *et al.*, 1995). Elevated CO_2 in the atmosphere can also affect soil CO_2 flux (Harper *et al.*, 2005), soil N deposition and decomposition (Van Kessel *et al.*, 2006), soil organic matter storage (Pendall *et al.*, 2011), and C and N cycling (Dijkstra *et al.*, 2010; Pendall *et al.*, 2011). Besides its effects on soil and nutrient dynamics and availability, elevated atmospheric CO_2 concentrations also interacts with above factors to cause short-term responses and long-term consequences in plant growth. Increasing atmospheric CO_2 concentrations can not only directly affect vegetation, and also have potential biological impacts from CO_2-induced warming and altered precipitation (Nowak *et al.*, 2004).

Species sensitivities to CO_2 are driven by different photosynthetic metabolisms, water use efficiency (Morgan *et al.*, 2004b), seed production, and seedling recruitment (Morgan *et al.*, 2004a). C_3 plants usually achieve greater productivity under elevated atmospheric CO_2 concentrations due to directly stimulated photosynthesis (Sage & Kubien, 2003) and indirectly increased soil moisture (Owensby & Ham, 1999; Morgan *et al.*, 2004b). C_4 plant growth, on the other hand, responds positively to warming (Sage & Kubien, 2003; Luo, 2007) and neutrally to positively to elevated CO_2 concentrations because of more favorable water relations (Morgan *et al.*, 2001; Sage & Kubien, 2003). The adaptation and evolution of relatively modern species and ecosystems, such as C_4 grasslands, which have evolved under low atmospheric CO_2

concentrations compared with contemporary concentrations, will especially be affected by the recent and rapid changes in atmospheric CO_2 (Morgan *et al.*, 2007). Elevated atmospheric CO_2 concentrations will benefit legumes more than forbs and grasses (Teyssonneyre *et al.*, 2002), especially in N- limited environments (Warwick *et al.*, 1998), while warming will favor shrubs over forbs (Harte & Shaw, 1995). Species-specific responses as well as different responses to elevated CO_2 concentrations and warming among plant groups can cause changes in species composition and diversity, thereby potentially affecting the structure and function of natural ecosystems (Potvin *et al.*, 2007).

2.1.2 Mixed-grass Prairie

Grasslands, savannas and shrub steppe constitute more than 40% of the Earth's terrestrial surface (Campbell & Stafford, 1997; Polley, 1997; Chapin *et al.*, 2001). Although rangelands are often characterized by water or nutrient limitations (Polley, 1997), they sustain domestic livestock grazing, one of the world's most extensive agricultural practices (Noble & Gitay, 1996; Squires & Sidahmed, 1997; Polley *et al.*, 2000). Rising atmospheric CO_2 concentrations and predicted global warming are expected to have substantial impacts on grasslands (Morgan *et al.*, 2004b; Niklaus & Körner, 2004). Their rapid responses to changes in climate can have dramatic ecological and social consequences(Lauenroth *et al.*, 1999).

The Mixed-grass Prairie of central North America was named by Weaver and Clements (1938) because of its two-tiered canopy structure. Lauenroth *et al.* (1999) combined and modified the grassland maps of Dodd (1979) and Küchler (1964), and categorized the vegetation of the central North America into three grassland types including Tallgrass Prairie, Mixed-grass Prairie, and Shortgrass Prairie. These grassland types were based on the seasonality and the photosynthetic pathways of dominant species (Küchler, 1964; Dodd, 1979; Lauenroth *et al.*,

1999). The Mixed-grass Prairie, located west of the Tallgrass Prairie and northeast of the Shortgrass Prairie, occurs in the central and north portion of the Great Plains, extending from southern and central Texas in the United States to southeastern Manitoba, Saskatchewan, and central Alberta in Canada (Wickens, 1998; Lauenroth *et al.*, 1999). Lauenroth *et al.* (1999) further divided the Mixed-grass Prairie into northern and southern types. The Northern Mixed-grass Prairie has a mixture of C_3 mid-grasses and C_4 shortgrass species. The mid-grass and shortgrass co-dominants in the Southern Mixed-grass Prairie are C_4 species (Lauenroth *et al.*, 1999).

Grasslands are composed of many species (Crous *et al.*, 2010) with extensive diversity in growth form, seasonal dynamics, major photosynthetic pathways, and longevity (Sims *et al.*, 1978). Two major photosynthetic pathways, namely C_3 (Benson-Calvin) and C_4 (Hatch- Slack), generally corresponding with cool-season and warm-season response, respectively, are represented among the species on the Mixed-grass Prairie. A typical example of C_3 plants is the cool-season grass *Pascopyrum smithii* (Rydb.) A. Löve, with the warm-season grass *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths as an example of C_4 plants (Williams III & Markley, 1973; Williams III, 1974). C_3 grasses grow mostly in spring while C_4 grasses grow in summer, together providing temporal displacement in growth (Williams III & Markley, 1973; Ode *et al.*, 1980a; Ode *et al.*, 1980b). Primary productivity is dominated by C_3 grasses in the spring, then shifts to C_4 grasses during the summer (Ode *et al.*, 1980a; Ode *et al.*, 1980b; Barnes *et al.*, 1983; Monson *et al.*, 1983; Boryslawski & Bentley, 1985; Paruelo & Lauenroth, 1996; Tieszen *et al.*, 1997). Generally, there are three to four times more forb species as compared with grasses, as well as annuals compared with perennials, whereas shrubs, half-shrubs, and trees are not common (Sims *et al.*, 1978).

The Northern Mixed-grass Prairie covers an area about 270,000 km^2. Situated between the Shortgrass and Tallgrass Prairie, the Northern Mixed-grass Prairie possesses intermediate growing season length, vegetation structure, and rainfall compared with the drier Shortgrass Prairie to the west, and the moister Tallgrass Prairie to the east. The mean annual temperature, average summer and winter temperatures are approximately 3°C, 15.5°C, and -10°C, respectively, with annual precipitation averaging from 325 to 450 mm. The moisture conditions in this ecoregion are considered as semiarid (Lauenroth *et al.*, 1999).

Grasslands in North America are of great importance. In the U.S., about 50%, or 400 million hectares is rangeland. Grasslands as the main component of rangelands not only compromise a large portion of the land area, but also provide natural pastures for domestic animals, which are one of the essential food sources (Sims *et al.*, 1978). However, after European settlement in the mid-19th century, more than 75% of the Northern Mixed-grass Prairie has been altered, with agricultural conversion as the primary purpose (Shay *et al.*, 2001). The current land use on the US and Canadian portion of the Northern Mixed-grass Prairie is slightly different. The US portion still has a large area in native grasslands, especially in western Nebraska and South Dakota, southeastern Montana, southwestern North Dakota, and eastern Wyoming. In the Canadian portion, a large fraction has been converted to farmland, with most of the remaining native vegetation grazed by cattle (Lauenroth *et al.*, 1994a).

Natural grasslands can offer a wide range of ecological goods and services. Native prairie provides forage for grazing ungulates, wildlife, birds and insects, as well as habitats for large and small mammals, birds, insects, reptiles and amphibians. The vast spatial, genetic, and biological diversity it possesses provides high quality nutrition to herbivores. Natural grasslands act as a major carbon sink and store large amounts of carbon in the soil. Besides the function of carbon

sequestration, various legumes and certain non-leguminous herbs on the rangelands can perform nitrogen fixation, helping with nitrogen deficiency during plant growth. The vegetative cover protects soil from water and wind erosion and degradation, traps sediment, and sustains the massive carbon sink of temperate grassland soils. The native prairie also functions as a filtration and neutralizer system, storing and managing water-flow patterns, and recycling nutrients. The open space it provides for humans, mammals, birds and insects often gives human being a sense of refuge and peace from a tiring, stressful world, an opportunity to reflect about nature and self, and an occasion to perceive non-monetary values and inner peace (Bailey *et al.*, 2010). All these benefits derived from the ecological function of natural grasslands are essential to life on Earth and contribute directly and indirectly to human welfare and economic functioning. Global climate change, characterized by increasing atmospheric CO_2 concentrations and projected warming, has had and will have substantial impacts on native grasslands, leading to further ecological, economic, and social consequences (Lauenroth *et al.*, 1999; Morgan *et al.*, 2004b; Niklaus & Körner, 2004).

2.1.3 Mixed-grass Prairie under global climate change

As the only carbon source for plants, rising atmospheric CO_2 concentrations could directly affect plant growth. Elevated atmospheric CO_2 concentrations increased growth of individual grass, sedge, legume, and forb species (Curtis *et al.*, 1989; Warwick *et al.*, 1998; Morgan *et al.*, 2004a). Increased phytomass under CO_2 enrichment had been reported in Calcareous grassland (Warwick *et al.*, 1998), C_4-dominated Tallgrass Prairie (Owensby *et al.*, 1993), Shorgrass Steppe (Hunt *et al.*, 1996), and semiarid Mixed-grass Prairie in Colorado (Parton *et al.*, 2007). CO_2-induced enhancement in production occurred above- and below-ground. Greater shoot mass and faster tiller production were observed under CO_2 enrichment

(Sionit *et al.*, 1980; Warwick *et al.*, 1998; Derner *et al.*, 2003). Root length, growth rate, root dry weight, and phytomass could all be increased by elevated CO_2 concentrations (Curtis *et al.*, 1989; Warwick *et al.*, 1998; Wechsung *et al.*, 1999); (Owensby *et al.*, 1993; Jongen *et al.*, 1995). CO_2 enrichment may also change root morphology and plants allocate more mass to lower depths of the soil profile (Wechsung *et al.*, 1999; Derner *et al.*, 2003).

Nutrient limitations may reduce the positive effect of CO_2 enrichment on plant growth. In frequently- disturbed, water-, and N- limited Calcareous grasslands, elevated CO_2 concentrations increased the mass and abundance of legume species, but did not affect that of other forbs and grasses (Warwick *et al.*, 1998). In N-limited, C_3, temperate grasslands, CO_2 enrichment increased the portion of forbs and legumes, but decreased the portion of grasses (Teyssonneyre *et al.*, 2002). When N was not limited, positive phytomass responses were observed in both C_3 grasses and perennial forbs in grasslands (Crous *et al.*, 2010). N-fixing ability appears to be important for species more responsive to elevated CO_2 concentrations and to gain a competitive advantage, especially in N-limited ecosystems (Warwick *et al.*, 1998).

Atmospheric CO_2 enrichment reduces transpiration because of partial closure of stomatas. On the other hand, it is also a plant fertilizer which can increase leaf area, thereby increasing plant transpiration. Parton *et al.* (2007) forecasted increased soil water content and soil respiration under elevated CO_2 concentrations in a Mixed-grass Prairie with the DAYCENT model (Parton *et al.*, 2007). The improved soil and plant water relation caused by elevated CO_2 concentrations could extend the growing season by either delaying the final days of the growing season (Chiariello & Field, 1996; Field *et al.*, 1997; Fredeen *et al.*, 1997; Grünzweig & Körner, 2001b; Lund, 2002), or by increasing the effective growing season with a decreased number of periodic drought days throughout the growing season (Morgan *et al.*, 2004b). Since rising CO_2

concentrations have multiple, positive effects on plant water relations, it may most probably increase plant production on moderately water-limited and disturbed rangelands when nutrients are not limited (Polley, 1997). CO_2-induced production increases progressively as ecosystems become drier (Polley *et al.*, 2000). Doubled atmospheric CO_2 concentrations increased the production of perennial grasses in Shortgrass Steppe (Hunt *et al.*, 1996). It also increased the production in C_4-dominated Tallgrass Prairie during dry years, but not during wet years (Owensby *et al.*, 1993). Enhanced water relations were a crucial feature in the CO_2 phytomass responses in Tallgrass Prairie in Kansas, Shortgrass Steppe in Colorado, and Calcareous grassland in Switzerland(Morgan *et al.*, 2004b). High CO_2 concentrations enable plants to better compensate soil-water-stress conditions when water begins to limit growth and positive effects of elevated atmospheric CO_2 concentrations on water relations are expressed (Sionit *et al.*, 1980; Wechsung *et al.*, 1999).

Elevated atmospheric CO_2 concentrations can also affect some slow-changing processes, such as soil N availability and C and N cycling. Enriched CO_2 concentrations increased soil C storage in C_4 vegetation through increased growth with less decomposition, but did not affect soil C storage in C_3 vegetation in native, Australian temperate grasslands (Pendall *et al.*, 2011). The total amount of soil C and N in the 0-75 cm depth was not significantly changed after 10 years of atmospheric CO_2 application in a fertilized and regularly cut pasture system (Van Kessel *et al.*, 2006). De Graaff *et al.* (2006) conducted a meta-analysis of CO_2 enrichment studies in a broad range of ecosystems; elevated CO_2 concentrations did not affect gross and net N mineralization, but microbial N immobilization increased. However, in water-limited semiarid grasslands, Dijkstra *et al.* (2008) reported increased rates of N mineralization and plant N uptake, with decreased nitrogen oxide (NO_x) gas loss under CO_2 enrichment, indicating a more closed N

cycle. A reduced soil inorganic N pool due to increased microbial N immobilization under elevated CO_2 concentrations (Dijkstra *et al.*, 2010) may lead to increased retention of N in long-lived plant biomass and soil organic matter, reducing soil N availability, and ultimately restraining plant growth (Luo *et al.*, 2004; Reich *et al.*, 2006). Those changes acted together with CO_2 enrichment to affect plant productivity and C sequestration with short-term, rapid-changing effects and long-term effects.

Differential plant species responses and functional group sensitivities to elevated atmospheric CO_2 concentrations can cause changes in species composition and diversity, potentially affecting the structure, function, and dynamics of natural ecosystems (Morgan *et al.*, 2007; Potvin *et al.*, 2007). When N was limited, enriched CO_2 concentrations increased the phytomass and relative abundance of legumes, but decreased that of grasses, with positive to neutral effects on that of forbs in a temperate C_3 grassland (Teyssonneyre *et al.*, 2002) and a Calcareous grassland (Warwick *et al.*, 1998). However, when N was not limited, or with N application, elevated atmospheric CO_2 concentrations reduced the photosynthetic capacity, phytomass, and relative abundance of C_3 forbs. Contrary to the N limitation situation, the phytomass and photosynthetic performance of C_3 grasses were enhanced under CO_2 enrichment (Crous *et al.*, 2010). C_4 grasses usually responded less to elevated CO_2 concentrations when compared with rapid-growing plants, woody plants, and cool-season grasses (Polley, 1997). Plant species diversity, evenness, and richness were not significantly affected by doubling CO_2 concentrations in the Shortgrass Steppe in Colorado for five years (Morgan *et al.*, 2007). However, the CO_2-induced enhancement in growth of *Hesperostipa comata* (Trin. & Rupr.) Barkworth ssp. *comata* and *Artemisia frigida* Willd. decreased similarity between communities over the five years. The cover of C_3 grasses, forbs, and shrubs increased while that of C_4 grasses

declined under elevated CO_2 concentrations. Different responses among species and functional groups to rising atmospheric CO_2 concentrations could lead to further changes in the composition, function, and dynamics of rangeland vegetation.

CO_2-induced warming, altered precipitation regimes, and improved water relations interact with elevated atmospheric CO_2 concentrations to affect natural ecosystems at different levels. Coughenour and Chen (1997) reported that warming alone decreased plant productivity, but productivity increased when combined with elevated CO_2 concentrations, while Melillo *et al.* (1993) predicted positive responses in plant productivity to elevated CO_2 concentrations and warming, but with smaller magnitude in warming (Melillo *et al.*, 1993). Pepper *et al.* (2005) and Parton *et al.* (2007) found the same increasing effects of elevated CO_2 concentrations and warming on productivity with their combination giving the greatest response (Pepper *et al.*, 2005; Parton *et al.*, 2007). C_4 plants responded more to warming in growth while C_3 plants usually achieved enhanced productivity under elevated CO_2 concentrations (Sage & Kubien, 2003; Luo, 2007). In C_4 vegetation on a native, Australian temperate grassland, warming alone increased soil C storage, but not when combined with enriched atmospheric CO_2 concentrations. In a C_3 vegetation, neither treatment had significant effect on soil C storage (Pendall *et al.*, 2011). Elevated CO_2 concentrations and warming also had contrasting effects on the N cycle in a Mixed-grass Prairie. Enriched atmospheric CO_2 concentrations increased microbial N immobilization therefore decreased the soil inorganic N pool, while warming increased the soil inorganic N and plant N pool because of increased gross and net N mineralization. There were no interactive effects observed between elevated CO_2 concentrations and warming on N cycling in the semiarid grasslands (Dijkstra *et al.*, 2010).

Elevated atmospheric CO_2 concentrations can cause changes in the amounts and timing of rainfall events. Decreased productivity and seasonal soil CO_2 flux in native Tallgrass Prairie were observed both under reduced rainfall amounts and altered rainfall timing, with the greatest response occurring when the two factors were combined (Harper *et al.*, 2005). Also, changes in the timing of rainfall events may be as important as that in the amount of rainfall in affecting soil CO_2 flux and other components of the C cycle in native North American grassland ecosystems. Percolation of water saved under elevated CO_2 concentrations to lower soil depths may open niches for deeper-rooted species (Polley *et al.*, 1997; Nelson *et al.*, 2004) and alter competition among plant species for soil water, thus having important consequences on plant community dynamics. However, this CO_2-induced increase in deep drainage was only found in wet years in Shortgrass Steppe in Colorado and Tallgrass Prairie in Kansas. In more mesic ecosystems, when available soil nutrients are in low supply, CO_2-induced improved water relations will likely increase the rates of decomposition and N mineralization, therefore increasing nutrient availability, resulting in manifest photosynthetic responses (Morgan *et al.*, 2004b).

Elevated atmospheric CO_2 concentrations can affect leaf N concentrations and forage quality (Wand *et al.*, 1999; Morgan *et al.*, 2004a). C_3 forbs possessed lower capacity in soil resource exploitation and N acquisition than C_3 grasses to maintain leaf N under elevated CO_2 concentrations and N addition (Crous *et al.*, 2010). However, when compared with C_4 grass species, C_3 species responded negatively to CO_2 enrichment in forage quality. Elevated CO_2 concentrations increased sugar, starch, and fructan contents, and decreased the protein level of C_3 grasses, but had little effect on those of C_4 grasses (Barbehenn *et al.*, 2004). Although Barbehenn *et al.* (2004) believed that the reduced quality of C_3 grasses was still higher than that of C_4 grasses, Morgan *et al.* (2004b) found that enriched CO_2 decreased the digestibility of *Bouteloua*

gracilis, *Pascopyrum smithii*, and *Hesperostipa comata*, the three dominant species of the Shortgrass Steppe in Colorado.

Vasseur and Potvin (1998) reported that CO_2 contributed the most among all environmental factors and became increasingly important over time in affecting the species composition of a natural pasture community. However, the positive effects of elevated CO_2 concentrations on plant growth and water relations may not be scalable to community or whole landscape levels (Warwick *et al.*, 1998; Morgan *et al.*, 2004b). It may not be accurate to predict community-level response or even species response within a community based on the responses of individual species to elevated atmospheric CO_2 concentrations because they are affected both by direct physiological effects and indirect competitive effects (Warwick *et al.*, 1998). CO_2-induced, indirect, water-driven responses may not be applicable to higher spatial and longer temporal scales, because atmospheric feedbacks may alter atmospheric humidity through widespread stomatal and transpirational responses, and may lessen responses observed in small experimental plots (Morgan *et al.*, 2004b). Moreover, species abundances on rangelands are determined more by their morphological and phenological features and responses to disturbances rather than by physiological attributes that are more responsive to CO_2 concentrations (Polley, 1997). Also, plant production on rangelands is often constrained by water or nutrient limitation (Medina, 1982; McNaughton *et al.*, 1988; Seastedt *et al.*, 1991), which may reduce the absolute positive effects of elevated CO_2 concentrations on rangeland vegetation. Atmospheric CO_2 can directly affect plant performance, but such influence, together with other factors, interacts with plant physiology, growth form, and life-history characteristics to affect plant-plant interactions. If other factors such as temperature and frequency of disturbances change, the relative

importance of CO_2 to species dynamics and ecosystem structure and function may also vary (Polley, 1997).

2.2 Reproduction in grassland as affected by global climate change

Studies on the effects of elevated atmospheric CO_2 concentrations on plants have focused primarily on the vegetative phase and have been conducted on many different species and ecosystems (Poorter, 1993&, 1998; Poorter & Nagel, 2000; Poorter & Pérez-Soba, 2001). Functional groups of plants that have shown consistent and distinctive vegetative responses to elevated CO_2 concentrations are reflected in photosynthesis and nutrient utilization (Poorter, 1993; Wand *et al.*, 1999). Physiological changes under enriched CO_2 concentrations such as reduction in leaf nitrogen concentrations, accumulation of leaf starch, and down regulation of photosynthesis (Stitt & Krapp, 1999; Körner, 2000) may affect carbon and nitrogen supply available to reproductive organs (Lawlor, 2002) and in turn constrain the magnitude of potential responses to elevated CO_2 concentrations during this phase of the life cycle. Plant reproduction responses to rising atmospheric CO_2 concentrations have important implications in natural (Körner *et al.*, 1996) and agro-ecosystems (Wittwer, 1995; Bazzaz & Sombroek, 1996; Rosenzweig & Hillel, 1998). Changes in plant reproductive success could further alter the composition and hence the functioning of natural plant communities (Bazzaz & Sombroek, 1996; Grünzweig & Körner, 2001a).

Flowering time responses to elevated atmospheric CO_2 concentrations can be species-specific. CO_2 enrichment delayed floral initiation and flowering in sorghum (*Sorghum bicolor* (L.) Moench) (Hesketh & Hellmers, 1973) and Johnsongrass (*Sorghum halepense* (L.) Pers.) (Carter & Peterson, 1983) but advanced that of annual phlox (*Phlox drummondii* Hook.) and Jimsonweed (*Datura stramonium* L.) (Garbutt & Bazzaz, 1984), and had no effect on those of

wheat (*Triticum aestivum* L.) (Krenzer & Moss, 1975) and velvetleaf (*Abutilon theophrasti* Medic) (Garbutt & Bazzaz, 1984). Elevated CO_2 concentrations increased seed yield of soybean (*Glycine max* (L.) Merr.) (Hardman & Brun, 1971). It also increased the grain yield of wheat through increased kernel number per plant and kernel size (Krenzer & Moss, 1975). Moreover, enriched CO_2 concentrations can compensate the negative effects of water stress and nutrient limitations on grain yield, number, and size of wheat (Sionit *et al.*, 1980; Sionit *et al.*, 1981), but it cannot completely offset the adverse influence of water stress (Sionit *et al.*, 1980). Increased root:shoot ratios under nutrient limitations have also been noted (Sionit *et al.*, 1981).

The effect of CO_2 enrichment, increasing temperature, and other parental conditions during plant growth and seed production, and its influence on seed germination have been reported in many species (Wulff & Alexander, 1985; Garbutt *et al.*, 1990; Andalo *et al.*, 1996). A meta-analysis on the effect of elevated atmospheric CO_2 concentrations on eight reproductive traits of 79 crop and wild species showed that CO_2 enrichment resulted in more flowers (+19%), fruits (+18%), seeds (+16%), individual seed mass (+4%), total seed mass (+25%), and lower seed nitrogen concentrations (−14%) (Jablonski *et al.*, 2002). Seed N was not affected by elevated CO_2 concentrations in legumes, but declined significantly in most non-legumes. Newton (1991) observed a trend towards greater seed production per plant and greater individual seed weight, but a variable response in seed numbers among nine pasture and old-field species at elevated compared with ambient CO_2 concentrations. Elevated CO_2 concentrations doubled the density and total seed production of wild oat (*Avena barbata* Brot.) during a dry year mainly by increasing seedling establishment and survival, the increase of which was much more than that in a relatively wet year (Jackson *et al.*, 1995). Greater production of legumes and forbs in a New

Zealand pasture was caused mainly by greater seed dispersal under elevated CO_2 concentrations (Edwards *et al.*, 2001).

Elevated atmospheric CO_2 concentrations had significant, positive effects on germination rate, seed production, and seed quality of paper birch (*Betula papyrifera*) (Darbah *et al.*, 2007). In a 5-year study on a natural, nutrient-poor Calcareous Grassland, Thürig *et al.* (2003) found a 42% increase in seed number of graminoids, a 33% increase in forbs, and no significant change in legumes (-38%) under elevated CO_2 concentrations. In most of the species, seeds tended to be heavier (+12%), and N-concentration of seeds was reduced in eight out of 13 species. Total germination did not differ, but the time to germination was significantly shortened under elevated CO_2 concentrations (Thürig *et al.*, 2003). Hovenden *et al.* (2008) studied the effect of warming, and the interactions between warming and elevated CO_2 concentrations on seed mass and germination of *Austrodanthonia caespitose* (Gaudich.) H.P. Linder. Mean seed production and mass were not significantly affected by either elevated CO_2 concentrations or warming, but elevated CO_2 concentrations more than doubled the proportion of very light, non-viable seeds, and halved mean seed N concentrations and N content. The dependence of seed germination on seed mass was affected by an elevated CO_2 concentrations × warming interaction, such that maternal exposure to elevated CO_2 concentrations or warming reduced germination if applied in isolation, but not when applied in combination (Hovenden *et al.*, 2008). In Australian temperate grasslands, elevated CO_2 concentrations and warming favored a dominant C_4 species (*Themeda triandra* Forssk.) over several C_3 species in seed germination, seedling growth, and mortality (Williams *et al.*, 2007; Hovenden *et al.*, 2008). Elevated CO_2 concentrations reduced seed N and vigor, and increased seedling mortality of *A. caespitosa*, a dominant C_3 grass in native Australian temperate grasslands (Hovenden *et al.*, 2008).

2.3 Interaction between native and invasive species in the Mixed-grass Prairie as affected by global climate change

Elements of global change include rising atmospheric CO_2 concentrations, climate change, increasing nitrogen deposition, increasing disturbance and fragmentation, and biological invasions (Dukes & Mooney, 1999). Those components can affect species distribution and resource dynamics in ecosystems by favoring groups of species that possess certain physiological or life history traits, and consequently interact with biological invasions. Invasive species are predicted to be favored by ongoing global change, both supported by experimental studies (Blumenthal *et al.*, 2008; Blumenthal, 2009; Lei *et al.*, 2012) and modeling approaches (Bradley, 2009; Sandel & Dangremond, 2012). However, the intricate relationship between global change and plant invasion, as well as the variable responses of invasive species to the multiple components of global change, will make it difficult to predict general responses of invasive plants (Bradley *et al.*, 2010).

Invasive, alien species share traits that enable their dominance and promote their invasion under global change (Dukes & Mooney, 1999; Bradley *et al.*, 2010). First, invasive species are capable of thriving in new climates and of shifting ranges quickly (Dukes & Mooney, 1999). Alien species share traits of short generation times, early succession, rapid growth, fast dispersal, wide range of distribution, and independence from mutualists, which give them the ability to succeed in novel environments (Thuiller *et al.*, 2007; Bradley *et al.*, 2010). Secondly, invasive species are well suited to environments with high resource availability (Davis *et al.*, 2000; Blumenthal, 2006; Blumenthal *et al.*, 2009). Global change, including increasing atmospheric CO_2 concentrations and N deposition, could directly increase plant resource availability, which may exacerbate invasion (Bradley *et al.*, 2010). Thirdly, some exotic species share attributes

associated with warmer temperature. Predicted global warming will therefore increase the dominance of exotic species (Sandel & Dangremond, 2012). Sandel and Dangremond (2012) found that traits of taller individuals, larger leaves and seeds, and greater leaf N content in exotic species compared with native species, are all associated with regions with higher temperature across the United States. They predicted that climate change would exacerbate plant invasion in California grasslands (Sandel & Dangremond, 2012). Furthermore, 14 of the 18 worst weeds in the world possess C_4 photosynthesis, most of which are dicots (Ehleringer *et al.*, 1997). In a disturbed habitat, C_4 species are more likely than C_3 species to comprise a major component of the invaders (Elmore & Paul, 1983). C_4 species generally have higher optimum temperature for photosynthesis, and therefore may profit more from warmer temperatures than C_3 species. Some C_4 weeds might expand their ranges which are currently restricted by cold temperatures and become problem species over larger areas under future warmer climate (Patterson, 1995).

As one of the manifestations of global change, climate change, which includes rising temperatures, altered magnitude and seasonality of precipitation, and changed frequency and intensity of extreme climatic events, will impact the physiological capacity and competitive capability of invasive species and native species (Bradley *et al.*, 2010). In Australian temperate grassland, warming decreased the growth and productivity of invasive species in relation to native species (Williams *et al.*, 2007). Altered timing and amount of annual precipitation increased the invasion, expansion, and dominance of invasive alien grasses in California serpentine grasslands (e.g., soft chess (*Bromus hordeaceus* L.)) (Hobbs & Mooney, 1991), in the transition zone between the Great Basin and Mojave deserts (Hunter, 1991), as well as in the Sonoran desert (e.g., buffel grass (*Cenchrus ciliaris* L.)) (Burgess *et al.*, 1991). Furthermore, seasonal changes in precipitation appear to be a more critical forecaster of plant invasion

(Bradley *et al.*, 2010). In a North American Mixed-grass Prairie, added snowfall increased the above-ground mass of *Centaurea diffusa* and *Linaria dalmatica* from 0.03 to 66 g m^{-2} and from 5 to 101 g m^{-2}, respectively (Blumenthal *et al.*, 2008). Greater summer precipitation had minor effects on the mass and density of those invasive forbs. On the contrary, spring irrigation (in the California grassland) and the fall and spring watering (in the Great Basin desert), but not winter watering, that promoted plant invasion (Miller *et al.*, 2006; Thomsen *et al.*, 2006). In South Africa, tree invasion was facilitated only by enhanced summer precipitation during warm months (Lei *et al.*, 2012). Different from the positive effects of increased precipitation on invasion, less variation in winter precipitation and drier summers would enable greater germination percentage and rate for seeds of *Centaurea solstitialis* (Hierro *et al.*, 2009). In addition, modeling results also supported the importance of seasonality changes in precipitation of the impacts on plant invasion (Bradley, 2009). The frequency and intensity of extreme weather events, such as heat waves, droughts and hurricanes are predicted to be more common under climate change (Diez *et al.*, 2012). Changes in the extent and magnitude of such events are expected to facilitate biological invasion by supporting the colonization of invaders and by reducing the resistance of native communities to invasion (Diez *et al.*, 2012). For example, in New Zealand, heat waves stimulated the growth of rapid-growing invasive C_4 annuals (White *et al.*, 2001). In South Africa, big rainfall events promoted tree invasion in savannah (Richardson *et al.*, 2000). Although the occurrence of extreme climatic events will be more frequent and intense, which may enhance the risk of plant invasion, the effects vary among different ecosystems and types of events (Diez *et al.*, 2012). Observations and experimental studies have provided extensive support to the facilitating effects of climate change on plant invasion. However, several model projections show a reduction in invasion risk under future climate in the U.S.A. (Bradley, 2009; Bradley *et*

al., 2009), in Australia (Beaumont *et al.*, 2009), and in South Africa (Parker-Allie *et al.*, 2009). In summary, climate change could exert the distribution and prevalence of invasive plant species. But whether it will facilitate or impede invasion is specific to species and (or) location, and also highly associated with the extent and seasonality of change (Bradley *et al.*, 2010).

As an important component of global change, rising atmospheric CO_2 concentrations is predicted to facilitate invasion through increasing availability of plant resources. CO_2 enrichment was expected to benefit native and invasive species through the direct "fertilization" effect (Davis *et al.*, 2000; Blumenthal, 2006; Blumenthal *et al.*, 2009). But such increase in plant resources availability favored invasive species over native species (Lei *et al.*, 2012), or there was less or no response in resident plants (Dukes *et al.*, 2011). Moreover, the interactions with other global change components seems to be more important in examining the impact of rising CO_2 concentrations on plant invasion. When combined with warming, invasive plants failed to show the positive reaction observed under CO_2 enrichment alone (Williams *et al.*, 2007). On the other hand, interactions with other species should also be considered when predicting the effects of CO_2 enrichment on plant invasions (Dukes & Mooney, 1999). Many invasive plant species showed positive responses to elevated CO_2 concentrations when grown individually or in monoculture (Smith *et al.*, 1987; Sasek & Strain, 1988&, 1991), but not when grown in polyculture or in diverse communities (Taylor & Potvin, 1997). In monoculture, CO_2 enrichment increased above-ground phytomass production, inflorescence production, and midday photosynthesis of yellow star-thistle (*Centaurea solstitialis* L.), a highly invasive species in California grasslands (Dukes, 2002). Similar responses were not observed when it was grown in a polyculture.

Another global change component, N deposition, is hypothesized to exacerbate invasions, especially in N-limited conditions (Bradley *et al.*, 2010). Greater plant mass and competitiveness in invasive species over natives were observed with N deposition in deserts (Brooks, 2003), grasslands (Blumenthal *et al.*, 2008), and tidal marshes (Tyler *et al.*, 2007). In Mediterranean grassland, nitrogen addition had a positive effect on growth of *Centaurea solstitialis*, with a more than two-fold increase in phytomass (Dukes *et al.*, 2011). N addition also promoted the growth and competitive ability of *Eupatorium adenophorum* in southwest China (Lei *et al.*, 2012). In a dry, rocky foothills grassland in Colorado, the phytomass and seed production of *Linaria dalmatica* were two times larger in response to N enrichment (Jamieson *et al.*, 2012). However, increased nitrogen availability and herbivore attack decreased the iridoid glycoside concentrations in *Linaria dalmatica*, indicating a lower level of plant defense (Jamieson & Bowers, 2012). Same as CO_2 enrichment, the impacts of N deposition on plant invasion also interact with other global change factors, which may be even more important in predicting the future impacts (Bradley *et al.*, 2010). N deposition increased the biomass and density of *Linaria dalmatica* when combined with added snow in the Mixed-grass Prairie (Blumenthal *et al.*, 2008). On the contrary, nitrogen addition alone, without increased precipitation, increased the growth rate of *Hesperis matronalis* (Hwang & Lauenroth, 2008). For *Holcus lanatus*, the combination of N addition and disturbance promoted the cover of this species on grasslands in Britain (Buckland *et al.*, 2001). The relative advantages brought by CO_2 enrichment and N deposition to invasive species might be mediated by other global change components. It is therefore important to consider the interaction between multiple global change factors when predicting their impacts on plant invasion (Bradley *et al.*, 2010).

Besides the above climatic and biotic alterations that have been induced by global change, changes in land use or land cover, and rising global horticultural trade, is another significant aspect of global change. Those anthropogenic modifications in landscape could increase disturbance and fragmentation, damage native species, and provide substantial resource pool for invasion to occur, all of which also lead to a decrease in biological diversity (Hobbs & Huenneke, 1992; Canham *et al.*, 1994; Burke & Grime, 1996; Bradley *et al.*, 2010). Global commerce, especially worldwide horticultural trade, increases invasion risks by introducing novel invasive species accidentally and intentionally (Lodge *et al.*, 2006; Bradley *et al.*, 2011). With the growing demand for drought-tolerant species and novel species that could grow well under warmer temperatures, new species may become invasive. Emerging global trade in horticulture combined with shifting demands will facilitate plant invasion (Bradley *et al.*, 2011).

2.4 Biological and ecological characteristics of species studied

Nine species from the Mixed-grass Prairie were studied in these studies. Four are native perennial grasses including *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths (blue grama), *Koeleria macrantha* (Ledeb.) Schult. (Junegrass), *Pascopyrum smithii* (Rydb.) A. Löve (western wheatgrass), and *Hesperostipa comata* (Trin. & Rupr.) Barkworth ssp. *comata* (needle- and-thread), two pairs of native/invasive species including *Chenopodium leptophyllum* (Moq.) Nutt. ex S. Watson (narrowleaf goosefoot)/*Salsola iberica* Sennen & Pau (Russian thistle), *Grindelia squarrosa* (Pursh) Dunal (curly-cup gumweed)/*Centaurea diffusa* Lam. (diffuse knapweed), and a common non-native invasive species, *Linaria dalmatica* (L.) Mill. (dalmatian toadflax). Among these species, there is a wide variety of taxonomic and ecological characteristics.

2.4.1 Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths

Bouteloua gracilis, a native perennial C_4 grass, is common throughout the Great Plains (USDA-NRCS, 2002a). It is a low growing, sod-forming bunchgrass with fibrous roots. Curved, mature seed heads resemble a human eyebrow, making it easy to identify. Each slender erect flower stalk has 2-5 spikes, on one side of which seeds are borne. *Bouteloua gracilis* starts growing in May or June with the onset of summer rain (Stubbendieck *et al.*, 1986) and flowers from July to September (Allison *et al.*, 1977; McGregor & Barkley, 1986). It can enter dormancy during droughts, but greens up quickly when precipitation is received (Cronquist *et al.*, 1997). Sufficient fall precipitation can lead to new growth and sometimes a second (Weaver & Albertson, 1956; Rauzi *et al.*, 1969) or third (Wasser *et al.*, 1982) flowering. *Bouteloua gracilis* grows well on sandy loam, sandy, or gravelly soils (Cronquist *et al.*, 1997), but not in wet, poorly drained soils (Stubbendieck *et al.*, 1997). *Bouteloua gracilis* does not tolerate shade, flooding, or acid soils (Wynia, 2007), but this warm season grass is very drought resistant and will out-compete taller grasses when moisture is low (Nature North, 2000). Once established, *Bouteloua gracilis* is very competitive and displacement resisting. It exhibits good drought, average salinity, and moderate alkalinity tolerances (Wynia, 2007). As a shortgrass, *Bouteloua gracilis* is important on Mixed-grass Prairies, especially when these are grazed by large herbivores, since it can withstand livestock grazing and trampling (Sims *et al.*, 1973) andits importance increases when the prairie is under grazing pressure (Sims *et al.*, 1978). It is also a good resource for high quality fall and winter forage (Weaver & Albertson, 1956).

Bouteloua gracilis can regenerate by means of rhizomes, seeds, or tillers (Weaver & Albertson, 1956; Coupland & Johnson, 1965). However, seedling emergence and recruitment requires a specific microenvironment (Hyder *et al.*, 1971; Van Der Sluijs & Hyder, 1974; Briske & Wilson, 1977). Seeds usually germinate and emerge rapidly in warm moist soil with sufficient

soil water and soil temperature ≥ 15°C in the top 4 cm. Seedlings have a very high risk of death at 6 to10 weeks of age if individual seedlings fail to initiate and develop an adventitious root system. The seminal root system developed at the initial stage has limited ability to provide enough water for leaf expansion during seedling growth (Briske & Wilson, 1980). Seedling establishment of *Bouteloua gracilis* requires sufficient water in the 0-30 cm layer for seminal root growth, and sufficient water in the near surface soil and throughout the profile for development of adventitious roots (Lauenroth *et al.*, 1994b). Because of its character of rare recruitment from seeds (Weaver & Albertson, 1956) and the observation of no natural reproduction of *Bouteloua gracilis* in north-central Colorado from 1935 to 1975 (Hyder *et al.*, 1975), the prevalent view was that species population was sustained by vegetative propagation and that *Bouteloua gracilis* was unable to recruit from seedlings in regions where the annual precipitation is less than 380 mm (Laycock, 1989&, 1991). However, Coffin and Lauenroth (1990) found that seed availability was also an important potential constraint, and Aguilera and Lauenroth (1993) identified competition from adult plants as an important limitation on seedling survival. Moreover, Lauenroth *et al.* (1994b) argued that soil texture accounted for the spatial variability in the probability of recruitment. Soils with high silt content were most favorable for recruitment while soils with either high sand or high clay contents had low probabilities of recruitment. Annual precipitation, especially the intraseasonal distribution, played an important role in the occurrence of recruitment events, which explained to a large extent the temporal variability in recruitment (Lauenroth *et al.*, 1994b). Seedling recruitment of *Bouteloua gracilis* is sensitive to soil water availability. The probability of the occurrence of vegetative propagation is highly correlated with disturbance rates (Coffin & Lauenroth, 1988). The above two points implied that climate change, particularly changes involving the amount or the effectiveness of

soil water, could have significant effects on future populations of *Bouteloua gracilis* (Lauenroth *et al.*, 1994b).

2.4.2 Koeleria macrantha (Ledeb.) J. A. Schultes

Koeleria macrantha, is a short, tufted, and erect, perennial C_3 grass which is 15 to 60 cm tall. Narrow, distinctly veined, light green to bluish green leaves form small clusters (Ogle *et al.*, 2006) and grow to about 18 cm in length (Gonzalves & Darris, 2008). Erect seed heads rise from the basal cluster, developing dense, pale green to purplish spikes. There is one seed head on each stem which slightly fluffs open during flowering (Simonin, 2000). Each spikelet has 2 to 4 flowers, with sharply pointed and shiny glumes (Gonzalves & Darris, 2008). Common in the Mixed-grass Prairie, *Koeleria macrantha* is one of the most widely distributed native grasses in North America, comprising 3 to 10% of the native grass over the entire Mixed-grass Prairie in Canada (Coupland, 1950) and presenting up to 5% of many native communities (Ogle *et al.*, 2006). As one of the first grasses to green-up, this cool-season grass begins its vegetative growth in early spring (Coupland, 1950), flowers from April to June (Looman, 1978), with seeds maturing in July or August (Romo, 2000b). After growth is completed, the grass goes dormant until fall or the following spring, with the occurrence of fall regrowth if soil moisture is sufficient (Gonzalves & Darris, 2008).

Koeleria macrantha grows under a wide range of environmental conditions, soils, and plant communities (Gonzalves & Darris, 2008). It is adapted to rangeland meadows, plains, mountain foothills, and open forestlands, commonly found along trails in the Mixed-grass Prairie. Growth of the grass is best on well-drained soils with a pH of 6.0 to 8.0 (Ogle *et al.*, 2006). *Koeleria macrantha* has poor tolerance to salinity, moderate tolerance to cold, heat, drought, and serpentine soils, and good tolerance to fire (Simonin, 2000). Once established, it

tolerates moderate grazing, even during the fall months if there is adequate moisture to promote regrowth (Gonzalves & Darris, 2008). *Koeleria macrantha* is very important in prairie revegetation after a fire, severe drought, or other disturbances (Ogle *et al.*, 2006).

Koeleria macrantha usually disperses slowly, and mainly via seed (Simonin, 2000). Seed production varies from year to year and seed mass is relatively small with 4,000,000 seeds/kg. It can produce seeds, but there is a large portion of empty seeds and the overall seed viability is low, usually less than 50% (Simonin, 2000).

2.4.3 Pascopyrum smithii (Rydb.) A. Löve

Pascopyrum smithii, a sod forming, native, perennial C_3 grass, has extensive and spreading rhizomes. It has coarse blue-green leaves with prominent veins. Singly arising or clustering stems grow from 30 to 90 cm tall, and the stiff erect seed spike is about 5 to 15 cm long (USDA-NRCS, 2002b). Known as one of the most common and best known native grasses in the Great Plains, *P. smithii* is most abundant in the 250 to 500 mm annual precipitation zones. It starts to green up in March or early April, 2 to 3 weeks before *Bouteloua gracilis,* and matures later in the growing season, around mid-July to August. This cool season grass goes dormant during summer and regrows in the fall when temperatures are cooler. Good moisture conditions benefit spring and fall growth, while nitrogen application may allow it to compete with warm season grasses (Ogle *et al.*, 2009).

P. smithii is adapted to a wide variety of soils. It can grow in moderate to high moisture content, saline to saline-sodic, and medium to fine textured soils, with a preference for heavier but well-drained soils (Ogle *et al.*, 2009). It has moderate shade tolerance, fair drought tolerance, and good cold tolerance. It can also tolerate spring flooding, high water tables, and substantial silt deposition. Once established, stands can resist heavy grazing. Established *P. smithii* stands

are very competitive with weedy species. This modestly aggressive species is not recommended to be seeded with invasive, introduced, species, but it is compatible with adapted native species (USDA-NRCS, 2002b).

P. smithii can provide high quality forage which is palatable to all classes of livestock and wildlife. However, it is usually not very suitable for range seeding since it does not germinate and establish well (Berdahl & Frank, 1998). *P. smithii* spreads mainly through rhizomes. It can also spread via seed distribution but the germination is poor and the slowly developed stands may not even exist on the establishment year. It spreads slowly and takes quite a few years to become steadily established. Fine stands are normally achieved by the end of the fourth or fifth growing season. Once established, it is hardy and long-lived. Seed production of *P. smithii* has been satisfactory under cultivation conditions. Average production of 34 to 68 and 68 to 135 kg per acre can be accomplished under dryland and irrigated conditions in > 350 mm annual precipitation areas, respectively (Ogle *et al.*, 2009).

2.4.4 *Hesperostipa comata* (Trin. & Rupr.) Barkworth ssp. comata

Hesperostipa comata, is an erect, densely-tufted native perennial C_3 grass, accounting for about 1% of the basal cover in the Mixed-grass Prairie (Clarke *et al.*, 1950; Coupland, 1950; Tannas, 1997). It reaches a height of 30 to 120 cm. It has narrow basal leaves with a length of 7 to 30 cm, and shorter and wider leaves along the stem. *Hesperostipa comata* possesses a rapid spring growth beginning in mid-April. It is one of the first plants to start growth in the spring. Flower stalks appear in mid-June and flowering begins 10 days later (Coupland, 1950; Pearson, 1965). Seeds ripen in mid-July and shatter about two weeks later (Tisdale, 1947). It has nodding, loosely spreading panicles with single-flowered spikelets (Magee, 2002). The slender pointed seeds have long twisted awns with a length of 10 to 15 cm sticking out from their tips. The awn

is straightened by the first moisture after the seed is shed and twists again after drying out, screwing the seed into the soil for germination. Like "spears", seeds of *Hesperostipa comata* are easily embedded in the pant legs and shoe laces of visitors (Nature North, 2011). This cool season grass goes dormant in early to mid-summer and can regrow in fall if there is adequate precipitation (Magee, 2002).

Hesperostipa comata has a broad range of adaptation to soil types, ranging from excessively drained sands to fine sandy loams to coarse gravelly loam soils. It can also grow on loam to clay loam soils in some habitats (Magee, 2002). The most abundant of this species appears in 170 to 400 mm rainfall zones, with occasional occurrence in areas receiving as low as 130 mm to as much as 600 mm annual precipitation.

As a bunchgrass, *Hesperostipa comata* reproduces vegetatively through tillering. Older plants accumulate more tillers and occupy a lager basal area (Morgan *et al.*, 2004a). As a native species, it can also spread via seed. Although *Hesperostipa comata* may spread into adjacent vegetative communities under ideal climatic and environmental conditions, the dispersing rate is considered slow (Magee, 2002). Due to the avoidance of grazing, it can produce large amounts of seeds and seed production has been rather successful under cultivated conditions. Average production of 45 and 68 kg per acre are predictable under dryland and irrigated conditions in > 400 mm annual precipitation areas, respectively. *Hesperostipa comata* seeds have a relatively high level of dormancy. Pre-chilling treatments of 30 to 60 days can induce a secondary dormancy, reducing seed germination to 37 and 68%, respectively (Scianna, 2005). However, germination will not be affected with no or 120 days of pre-chilling.

2.4.5 Chenopodium leptophyllum (Moquin-Tandon) Nuttall ex S. Watson

Chenopodium leptophyllum, is a native, annual C_3 forb with slender growth habit (Wikipedia, 2011). The erect stem can grow up to 60 – 80 cm tall (Wilken, 1993). The dusty fleshy leave may be the narrowest among those of all the *Chenopodium* speices (Burns, 1982). It flowers from July to October (Plant for a Future, 1996-2010) and fruits from September to November (Burns, 1982). Seeds are ovoid and black, with a diameter of 0.9-1.1 mm, ripening from August to October (Flora of North America Association, 2008).

Chenopodium leptophyllum is native to most areas of western North America, ranging from Alaska to Texas and northern Mexico, and further east into central Canada (Wilken, 1993). It is found at the 300 to 3400 m elevation (Flora of North America Association, 2008). *Chenopodium leptophyllum* prefers full sun and open dry habitat, and grows well on sandy and gravelly soils (Fernald, 1950; Wilken, 1993). It is commonly found along rivers and roadsides, on hillsides, in waste places, and other disturbed areas and fields (Burns, 1982), but usually does not tolerate shade (Plant for a Future, 1996-2010). Although *Chenopodium leptophyllum* usually grows in disturbed habitats along with many Eurasian weeds, it is a native species in Canada and continental U.S.. Such growth habitat, in addition to its resemblance to other *Chenopodium* species, however, makes it easily unnoticed so it could spread more widely than is currently observed (Burns, 1982).

2.4.6 Salsola iberica (Sennen & Pau) Botsch. ex Czerepanov

Salsola iberica, a weedy, annual, C_4 forb, is native to Eurasia (Wagner & Shannon, 1997; Wagner *et al.*, 1999), and has spread to a variety of places throughout the world. The densely branched, bushy plant grows to 15-120 cm or more in height, consisting of a taproot reaching 1 m in depth, and extensive lateral roots spreading out to 1.8 m (Halvorson & Guertin, 2003). This early succession species achieves significant growth late in the season and flowers

from July to October. Seeds are black, shiny, and snail-shaped, with a diameter of 1.5-2 mm. Seeds mature from August to November, followed by a short period of dormancy in the field (Range Plants of Utah website, 2010).

Salsola iberica grows in a diversity of habitats, ranging from water edge to semi-desert regions (Holm *et al.*, 1997). It is commonly found on disturbed grasslands and desert communities, or unoccupied sites, with prevalence in the semi-arid regions of western United States (Young, 1991). *Salsola iberica* can be found on any type of soil with preference for well-drained sandy to loamy soils (Stubbendieck *et al.*, 1992). It adapts to and favors dry, disturbed, sandy soils. It cannot tolerate compacted soils, which will significantly reduce germination (Rutledge & McLendon, 2002). *Salsola iberica* can grow on dry to moist soils and does well on neutral and saline soils with a wide pH range of 7.0 – 7.9 (Duke, 1978&1979). This xerohalophytic forb also has very high water use efficiency, producing more biomass per unit of water used than most plants (Young *et al.*, 1995). Although *Salsola iberica* is competitive, it has high sensitivity to site conditions such as shade and crowded cultivation which constrain its establishment and success (Young, 1991). Germination of seeds is very vulnerable to molds and fungi attack and destruction. Its population may diminish with time after establishing because of mycorrhizal fungi causing autotoxic in the topsoil (Rutledge & McLendon, 2002).

Salsola iberica reproduces by seed and is a highly effective reproducer. According to different observations, seed production varies from 1,500 – 2,000 seeds/plant for a plant up to 0.5 m in height (California Department of Food and Agriculture, 2002), to 25,000 seeds/plant for a well-developed plant growing under little competition (Stevens, 1932), and up to 150,000 – 200,000 seeds/plant for an undisturbed plant (Young *et al.*, 1995). The fully differentiated, spirally coiled seed of *Salsola iberica* contains chlorophyll in the visible embryo, which enables

seeds to take advantage of short periods of favorable conditions and become established (Young *et al.*, 1995). Germination can be either epigeal or hypogeal (Howard, 1992). Seeds of *Salsola iberica* go dormant after maturity in fall and all through the winter months until spring. Based on this, Young *et al.* (1987) reported that fresh seeds will germinate under a narrow range of alternating day/night temperature regimes of 50/5°C, while in spring they can germinate across a wide range of temperature regimes of 50/-2°C. Germination can occur when night temperatures are below freezing and day temperatures reach 2°C, although seedlings remain very vulnerable to subsequent frost. Under optimum temperatures (7-35°C), adequate moisture (76 mm of rainfall), and loose soil conditions, *Salsola iberica* will germinate within a few hours (California Department of Food and Agriculture, 2002). Germination can occur in 38 minutes with a more restricted range of temperatures (7-10°C) (Rhods *et al.*, 1967). This fast-germination characteristic is very advantageous in a desert environment. Initiation of germination and rate of germination are positvely correlated with temperature. Young and Evans (1972) observed that germination was initiated in 2 hours and completed in 48 hours under constant temperature of 30°C. The seed itself can withstand several wetting and drying cycles and wait until there is sufficient moisture in the soil before emerging and establishing. At the same time, the wings attached to the seeds will help with moisture absorption from the soil (Howard, 1992). *Salsola iberica* can germinate very well with small amounts of on-site rainfall (3 mm) (Young *et al.*, 1995). Although germination is quick and fast, seedling establishment may fail if the soil is impacted or with crust, which prevents the root tips from penetrating the soil surface (Holm *et al.*, 1997). Also, cooler temperatures can significantly inhibit germination (Khan *et al.*, 2002). *Salsola iberica* has an inherent after-ripening requirement after seed maturity, which could severely limit the temperatures at which germination can occur (Young & Evans, 1972).

Therefore, germination is limited to very specific seedbed locations in some areas, and warm and wet autumns may be required for germination success in the fall (Khan *et al.*, 2002).

Salsola iberica has a unique mechanism for seed dispersal which greatly increases its spreading rate and infestation area. After seed maturity, the whole maternal plants are broken off and become tumbleweed, shattering seeds as they are blown by the wind along the surface of the ground. Seeds can be dispersed via animals, wind, water, and by human facilitation (Drezner *et al.*, 2001), and in contaminated crop seed (Holm *et al.*, 1997). Seed longevity seems to be short. Once in contact with the soil, 99% of the seeds either germinate or die within a year. However, seeds can remain viable for up to a year (Young, 1991), with rapid declining viability after 2 years in the soil (Young *et al.*, 1995).

2.4.7 *Grindelia squarrosa* (Pursh) Dunal

Grindelia squarrosa, a biennial to short-lived perennial forb, is native to most of areas from Manitoba south to Texas and west to Idaho and Arizona in the United States (Johnson & Nichols, 1970; Hitchcock & Cronquist, 1973). It forms a rosette in the first year, developing a large tough taproot with a widespread shallow root system and a short vertical rhizome (Weaver, 1968; Gleason & Cronquist, 1991). Extensively branched, semi-woody stems and flower heads grow and emerge in the second year (Donaldson & Mazet, 2011). The plant grows from 0.1 to 1 m tall (Barkley & McGregor, 1986). It flowers from June to September. The yellow flower is 1.5 to 7 cm wide, covered with abundant sticky resin, making the whole plant aromatic (Walsh, 1993).

Gridelia squarrosa is adapted to sandy loam, loam, and clayey loam soils, with fair growth on saline soils and poor to fair growth on gravel, clay, and dense clay soils. It generally favors drier sites but could also grow on moist soils when there is a lack of other vegetation

(Johnson & Nichols, 1970). It is most commonly found on roadsides, waste places, dry prairies, depleted rangelands, abandoned croplands, vacant lots, and other disturbed or unmanaged areas (Harrington, 1964; Seymour, 1982; Barkley & McGregor, 1986). It occurs throughout the western U.S., most commonly in the lower elevations of plains and foothills, as well as up to 3000 m in Colorado and New Mexico (McDonough, 1975). *Gridelia squarrosa* is capable of surviving and growing under adverse conditions, both from direct seedlings and seedling transplants (Nuzzo, 1976). However, it does not tolerate frequent disturbance (Donaldson & Mazet, 2011), thus usually colonizes longer-abandoned rather than the newly abandoned areas (Osborn & Allan, 1949). Poorly managed grazing promotes *Gridelia squarrosa*. It has an adverse economic impact on rangelands. However, it is rarely found on ranges in superior or good condition in the Central Great Plains (Klipple & Costello, 1960). Although is a good resource of food and nectar for pollinators in the late summer and early fall (Walsh, 1993), *Gridelia squarrosa* is unpalatable to livestock (Kaul & Keeler, 1980; Joyce & Morgan, 1989). It absorbs selenium from the soil and may concentrate to toxic levels for cattle (Bare, 1979). Native Americans have been using it for numerous medicinal purposes (Walsh, 1993).

Gridelia squarrosa reproduces by seed (Donaldson & Mazet, 2011). Seeds have pappus and can be dispersed by wind (Walsh, 1993). *G. suqarrosa* possesses seed polymorphism, with morphological dissimilarities between dis and ray achenes (McDonough, 1969). This morphological distinction requires different temperature optima and light conditions for germination to occur. Germination of disc achenes is faster and less light dependent than that of ray achenes. The different season and/or microsite requirements for germination occurrence would benefit the regeneration and seedling establishment of *Gridelia squarrosa*. It can either accomplish significant fall germination from disc achenes after being exposed to light and rapid

imbibition of water on the soil surface, or perform spring germination from ray achenes (McDonough, 1975).

2.4.8 *Centaurea diffusa* Lamarck

Centaurea diffusa, a semelparous, perennial, herbaceous plant (Thompson & Stout, 1991), is native to southeastern Europe and was introduced to North America around 1900 (Roche & Roché Jr, 1988; Lacey, 1989). It is considered to be an annual or biennial in Europe, a biennial in Pacific North America, and a short-lived perennial with rare occurrence observed by Keil and Turner (1993) (Keil & Turner, 1993). Highly branched stems can grow from 10 to 80 cm tall from a long, tough taproot. It forms a rosette after establishment and may stay in this form for one to several years until flower initiation, depending on plant size and vernalization (Thompson & Stout, 1991; Forest Health, 2006; Clements *et al.*, 2010; Thurston County, 2011). Urn-shaped white (occasionally pink to lavender) flowers are 3 mm in diameter and 1.4 to 1.6 cm high, appearing at the tips of the branches (Watson & Renney, 1974). There are two types of flowers: ray flowers around the edge, and the encompassing tube-shaped disk flowers. Small, dry achenes are 2 - 3 mm long, containing light brown to black seeds (Forest Health, 2006).

Centaurea diffusa grows at elevations from 150 to 900 m in Canada (Watson & Renney, 1974) and at elevations up to 2,600 m in the United States (Sheley *et al.*, 1998). It is commonly found along right–of–ways, dry forest edges, fringe forest, open forest areas, riverbanks, rangelands, and disturbed or overgrazed areas. It is adapted to light dry permeable soils but prefers semi-arid to arid conditions. It grows well in habitats ranging from open to shade areas (Forest Health, 2006).

Centaurea diffusa reproduces solely by seeds. It has the potential to produce large number of seeds per year (Clements *et al.*, 2010). A single flower stalk can produce up to 1200

seeds, and seed production of a whole plant can reach 18,000 seeds. After flowering from July to October, the plants dry out and break off at ground level, turning into tumbleweeds. Seed heads come off as the plants roll in the wind, travelling and dispersing over long distances (Forest Health, 2006; Thurston County, 2011). Seed production may vary greatly from year to year depending on moisture availability for plant growth (Sheley *et al.*, 1997). Only 0.1% of the annual seed rain is needed to maintain *Centaurea diffusa* stands (Schirman, 1981). Long seed dormancy and periodic germination also permit a large seed bank to establish (Thurston County, 2011).

As one of the most exotic, invasive species in western North America (Clements *et al.*, 2010), *Centaurea diffusa* had dispersed from 9 to 28 counties in the Pacific Northwest between 1950 and 1975 (Roché Jr & Talbot, 1986), inhabiting 1.2 million hectares in the western United States by the mid-1990s (Roche & Roché Jr, 1988; Lacey, 1989). It is very competitive and aggressive in rangeland plant communities, varying from severely grazed areas to those in high ecological condition (Lacey *et al.*, 1990). It crowds out native species, displaces wildlife and livestock forage, and competes for soil and water resources, reducing biodiversity and land value (Thurston County, 2011). *Centaurea diffusa* is comprised of allelopathic compounds. Once established, it forms dense colonies and monotypic stands, suppressing the growth of other species/vegetation (Forest Health, 2006).

2.4.9 *Linaria dalmatica* (L.) P. Miller

Linaria dalmatica, a short-lived, perennial C_3 forb, is native to southeastern Europe and southeastern Asian, and was introduced to North America as an ornamental plant around 1900 (Alex, 1962; Saner *et al.*, 1995). This invasive alien species now grows throughout the continental U.S. and in almost every province of Canada, being most abundant in western North

America ((Alex, 1962; Saner *et al.*, 1995; Vujnovic & Wein, 1997; Carpenter & Murray, 1998; Klein, 2011). It can grow up to 60 cm to 1.2 m tall, with alternating leaves clasping the glabrous erect stem (Klein, 2011). The two-lipped bright yellow snapdragon-like flower is 19 – 38 mm in length, including the extended spur borne on the upper branches of the stems (Whitson, 1991; Wetherwax, 1993; Royer & Dickinson, 1999). The fruits contain two-celled oval capsules, 5–7 mm in length, each of which has abundant small seeds. The seeds are black, abruptly angular, and slightly winged (Butler & Burrill, 1994; Council, 2012).

Linaria dalmatica is adapted to a wide range of soil types and moisture conditions and has good tolerance to low temperatures (Royer & Dickinson, 1999). It grows best in open sunny places on well-drained, coarse-textured soils with neutral to slightly alkaline pH (Vujnovic & Wein, 1997). It is commonly found on roadsides, waste areas, clear-cuts, overgrazed pastures and rangelands, and in plant communities that are open or have been disturbed (Carpenter & Murray, 1998).

Linaria dalmatica can reproduce sexually by seeds and vegetativly by horizontal or creeping rootstocks. Regeneration can also occur from root fragments as short as 1.3 cm (Zouhar, 2001). It has the potential to produce large numbers of seeds. Seed production can reach up to 500,000 seeds per mature plant per year (Carpenter & Murray, 1998). Most seeds fall to the ground within a short distance of the parent plants. Those that fall onto encrusted snow can be blown by the wind (Zouhar, 2001) or dispersed by animals for longer distances (Robocker, 1970; Vujnovic & Wein, 1997). Germination mainly occurs in spring, with some happening in fall. Seeds can remain dormant for up to 10 years. New infestations are usually initiated from seeds (Klein, 2011). There may not be a serious occurrence at the beginning because a single plant has little impact but this aggressive perennial can cause disastrous problems because of its

potential to spread through an extensive root system or seeds (Butler & Burrill, 1994). Once established, *Linaria dalmatica* can outcompete and replace native species, leading to further establishment and dominance of other invasive species in a community (Zouhar, 2001).

Chapter 3 MATERIALS AND METHODS

3.1 Heating, CO_2 enrichment, and irrigation in PHACE

The Prairie Heating and CO_2 Enrichment (PHACE) experiment was set up at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS), High Plains Grasslands Research Station (HPGRS), located about 16 km west of Cheyenne, WY, USA (41°11' N lat, 104°54' W long) with elevation of 1930 m in a Northern Mixed-grass Prairie (NMP) (Dijkstra *et al.*, 2010). The plant community is comprised of about 55% C_3 grasses with *Pascopyrum smithii* (Rydb.) A. Löve (western wheatgrass) and *Hesperostipa comata* (Trin. and Rupr.) Barkworth (needle-and-thread) dominating, C_4 grasses almost solely *Bouteloua gracillis* (Willd. ex Kunth) Lag. ex Griffiths (blue grama) contribute about 25% of the composition, and sedges, forbs and small shrubs contribute about 20% (Morgan *et al.*, 2011). *P. smithii* and *B. gracillis* comprise about 50% of the total aboveground biomass (Dijkstra *et al.*, 2010). Mean annual precipitation is 384 mm, and temperature average about 17.5°C in July and -2.5°C in January (Morgan *et al.*, 2011). The site, with an area of 2.4 ha, had been grazed by cattle from 1974 until 2004. The area was fenced in 2005 to preclude cattle grazing. The soil is a fine-loamy, mixed-mesic Aridic Argiustoll with the Ascalon series at the north side of the study site, and the Altvan series at the south side (Dijkstra *et al.*, 2010; Morgan *et al.*, 2011).

The PHACE experiment was initiated in spring 2006 and it ran until 2013 (Parton *et al.*, 2007). Thirty circular experimental plots (diameter 3.4 m) were established with each plot encircled by a 3.7-m-diameter plastic flange that was buried to a depth of 60 cm in the soil

(Figure 3.1) (Dijkstra *et al.*, 2010). The plastic barrier helped hydraulically separate treated plots from outside, non-treated soils (Morgan *et al.*, 2011). Each of the plots was split into two sections. One-half of the plot was retained as the Northern Mixed Prairie plant community and it was further divided into soil and plant sampling areas (NMP harvest area), plus sections for other measurements which included trace gas measurements, soil water content, air and soil temperature measurements, and a minirhizotron tube to study root responses. The other one-half of the plot was designed to study plant invasion. This area was comprised of three, 80 × 70 cm subplots. The Toadflax subplot was planted with 20 *Linaria dalmatica* (L.) P. Miller (dalmatian toadflax) seedlings in June 2006 and monitored until July 2010 to study its invasion into undisturbed Northern Mixed-grass Prairie (NMP). The other two subplots were partially disturbed to study the recruitment from seeds of species with different origins (native or exotic) and life histories (annual, biennial, or perennial), and the invasion and the ability of native species to recover from disturbance. Seeding was done twice - from 20-24 November 2007 and from 19-25 November 2008. Two, 80 × 20 cm strips were manually disturbed within each of the two subplots to a depth of 10 cm, killing native vegetation and mixing the soil. One 80 × 17.5 cm strip of native vegetation was bordered along the length to separate the two disturbed strips. Four pairs of native/invasive species were sown following the disturbance. Only species that grow locally in the Northern Mixed-grass Prairie (NMP) were selected, and the combinations were made based on life history and phylogeny (Table 3.1). The two sections within each plot were separated by a 25-cm-deep steel flange. The flange was oriented with the prevailing wind to minimize the potential influence of relatively tall invasive species on the CO_2 application in the native NMP side (USDA-ARS, 2010).

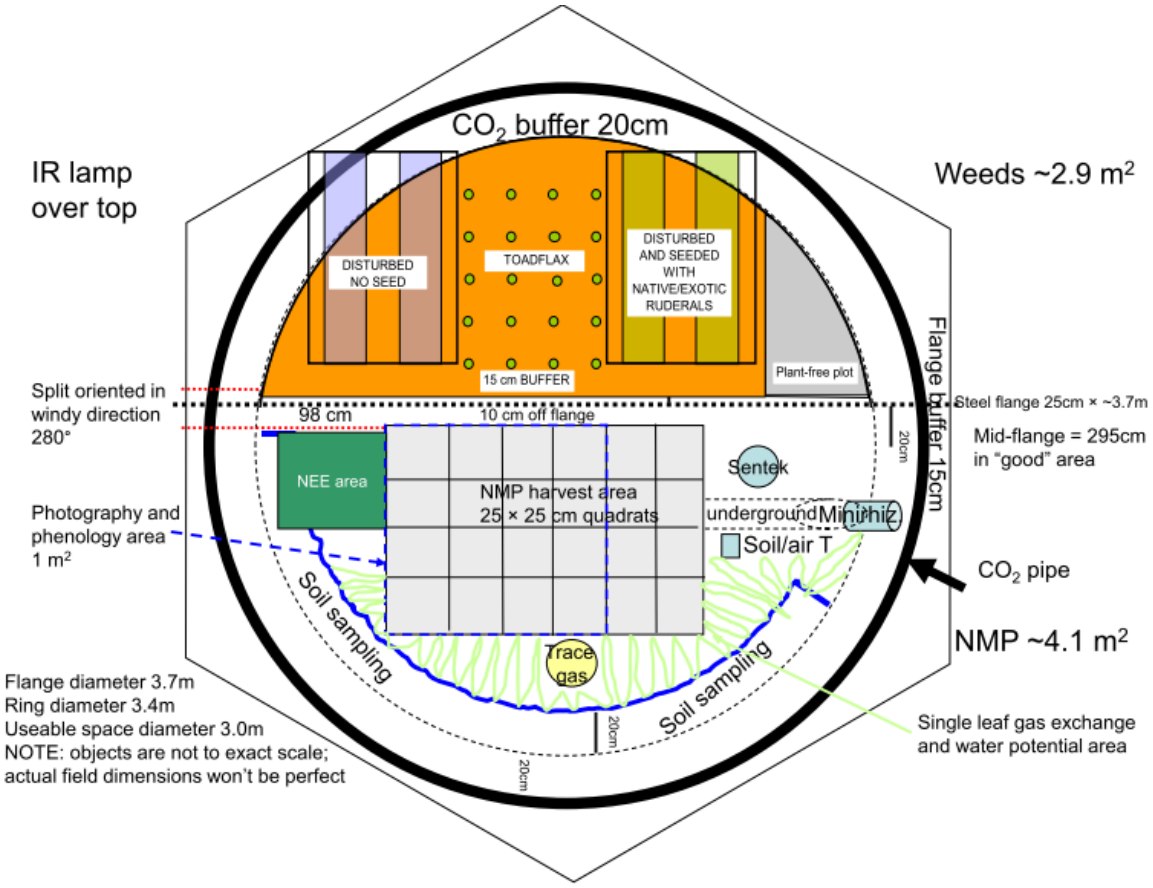

Figure 3.1 The Prairie Heating and CO_2 Enrichment (PHACE) experiment plot diagram (as seen from above), located in Cheyenne, Wyoming (Parton *et al.*, 2007; Kimball *et al.*, 2008).

Table 3.1 The four paired native/invasive species seeded inside the Prairie Heating and CO_2 Enrichment (PHACE) experiment plots, located in Cheyenne, Wyoming.

Life form	Native species	Invasive species
Annual	*Chenopodium leptophyllum*	*Salsola iberica*
Annual	*Descurainia pinnata*	*Descurainia sophia*
Biennial	*Grindelia squarrosa*	*Centaurea diffusa*
Perennial	*Artemisia frigida*	*Linaria dalmatica*

The PHACE experiment used Free Air CO_2 Enrichment (FACE) technology to elevate ambient CO_2 concentrations (Miglietta *et al.*, 2001), and infrared heater arrays to warm the canopy (Kimball *et al.*, 2008). Two concentrations of atmospheric CO_2 were applied including ambient (385 ppmv (c)) and elevated (600 ppmv (C)). Pure CO_2 was injected into the plots from a plastic pipe surrounding the plot, which had a line of laser-drilled, 300 µm holes with 15.9 mm

spacing. Pure CO_2 was pumped out at a pressure of about 1 mega Pascal, resulting in rapid mixing with ambient air and then dispersed into the plot by the wind. Each plot had an Infra-Red Gas Analyzer (IRGA) (PP Systems WMA-4) to monitor the CO_2 concentrations near the center of the plot, based on which a proportional valve array connected to the injection tubing was regulated (USDA-ARS, 2010). There were two temperature regimes, control (t) and heated (T) (1.5/3.0°C warmer day/night). Ceramic infrared heaters (1000 W; Mor Electric Heating Assoc., Inc., Comstock Park, MI, USA) were used to warm the canopy of the heated plots (Dijkstra *et al.*, 2010). A proportional-integral-derivative (PID) feedback system was used to sense canopy temperature, with a data logger installed to collect the radiometer data and run the PID control. Each heated plot had six infrared heaters fastened to a triangular frame, 1.5 m above the soil surface (USDA-ARS, 2010). The atmospheric CO_2 application was shut off during the winter and turned on in the spring in each experimental year (Table 3.2). The warming treatment began in April 2007 and ran all year round.

Table 3.2 Dates of turn-on and shut-off of atmospheric CO_2 application to the Prairie Heating and CO_2 Enrichment experiment plots between 2006 – 2009, located in Cheyenne, Wyoming.

	2006	2007	2008	2009
Turn-on atmospheric CO_2 application	28 Apr	10 Apr	2 Apr 2	31 Mar
Shut-off atmospheric CO_2 application	1 Nov	15 Nov	19 Nov	27 Dec

Twenty plots were used for the elevated CO_2 concentrations and warming treatments, consisting of a full factorial design with five replicates of each of the four combinations (ambient CO_2 and control temperature (ct); ambient CO_2 and heating (cT); elevated CO_2 concentrations and control temperature (Ct); elevated CO_2 concentrations and heating (CT)). Another ten plots were designated into two irrigation treatments with five replicates of each. Shallow irrigation (ct-s) was designed to simulate the effect of CO_2 on plant water use efficiency through frequent

water additions during summer, while deep irrigation (ct-d) was designed to imitate large precipitation events achieved by two irrigations, one in early spring and one in later summer (Table 3.3). The irrigation treatments started in April 2007. However, since it was very dry in 2006, eight irrigations of 20 mm each time were applied to all 30 plots during the summer (five in June, two in July, and one in August), increasing total annual precipitation by 72% in 2006 to 382 mm (Dijkstra *et al.*, 2010). There were a total of 30 plots on the study site (Figure 3.2).

Table 3.3 Water addition (mm) for the shallow and deep irrigation treatments in the Prairie Heating and CO_2 Enrichment (PHACE) experiment plots between 2007 – 2009, located in Cheyenne, Wyoming, USA.

Treatment	Plot	Year and amount (mm)		
		2007	2008	2009
Shallow irrigation	NMP harvest area	60	36	36
	Invasive species area*	24	24	24
	Total	84	60	60
Deep irrigation	NMP harvest area	60	36	36
	Invasive species area	40	24	24
	Total	100	60	60

* The Northern Mixed-grass Prairie (NMP) harvest area is larger than that of the Invasive species area. Therefore more water was added to the NMP side than the other side within each plot.

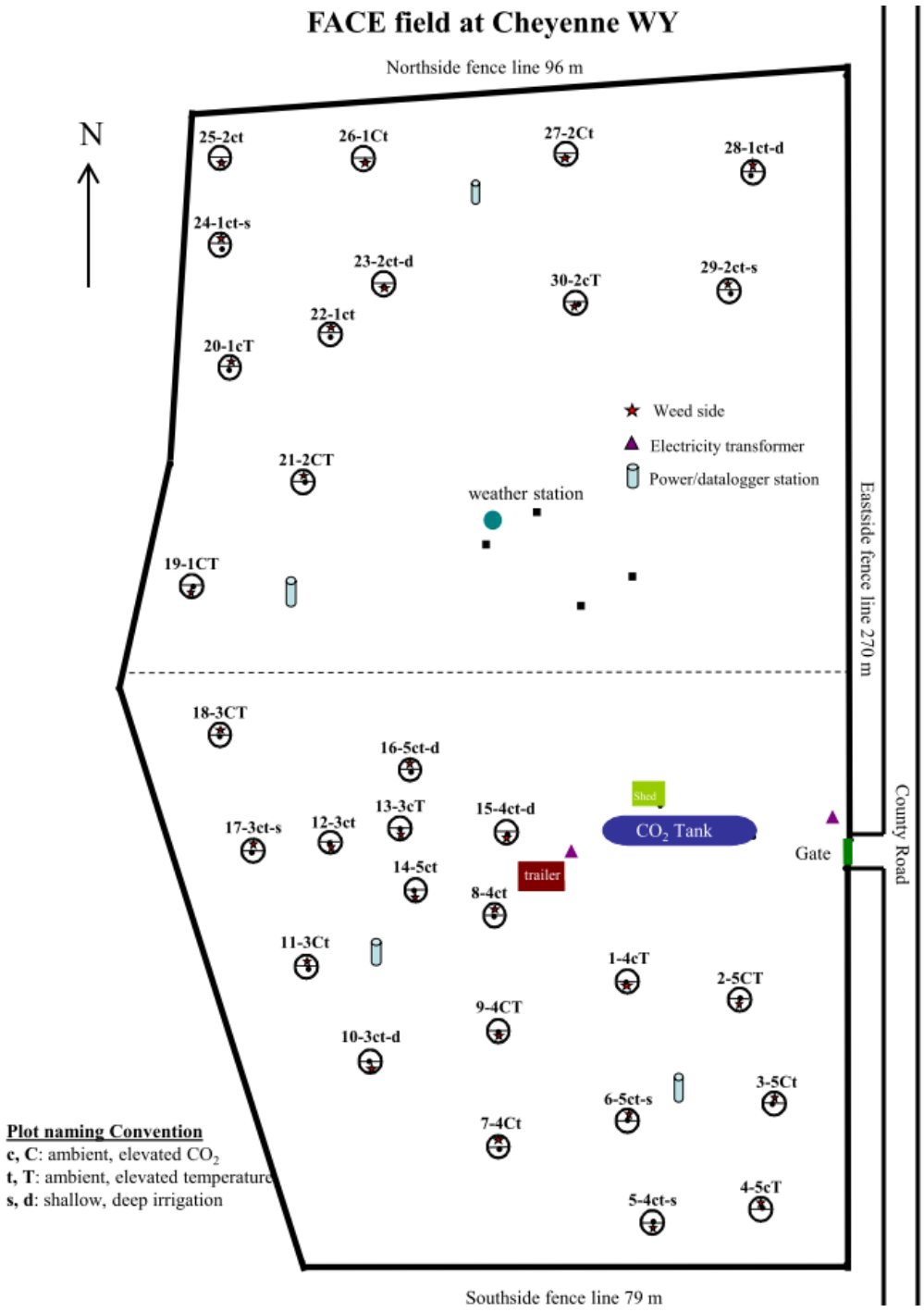

Figure 3.2 Field map of the Prairie Heating and CO_2 Enrichment (PHACE) experiment, located in Cheyenne, Wyoming, USA. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT) (Morgan *et al.*, 2011).

3.2 Seed materials and collection

Seeds of nine species were collected from both inside and outside the PHACE plots from 2007 to 2009 (Table 3.4). Seeds were given at least four weeks after-ripening at room temperature (about 20°C) before being stored in darkness at 4°C. The seeds were cleaned and the numbers of filled and empty seeds in each plot were recorded. A light table was used to separate the filled and empty seeds of *Koeleria macrantha* (junegrass) (Ledeb.) Schult..

Seeds of four native species including *Bouteloua gracilis*, *Koeleria macrantha*, *P. smithii* and *Hesperostipa comata*, a pair of native/exotic species *Chenopodium leptophyllum* (Moq.) Nutt. ex S. Watson/*Salsola iberica* Sennen & Pau, and one invasive species *Linaria dalmatica*, harvested from the outside of the plots in 2007 and 2008, were used to conduct the preliminary experiment. Seeds of the four native species were harvested from the Northern Mixed Prairie area inside each plot. Seeds of *Chenopodium leptophyllum*/*Salsola iberica,* and *Grindelia squarrosa* (Pursh) Dunal/*Centaurea diffusa* Lam., and *Linaria dalmatica* were harvested from the invasive species area of each plot.

3.3 Seed quality and germination tests

3.3.1 Individual seed mass

Dry seed mass of 10 to 30 seeds was determined for each species. Weighing was done with a CAHN C-32 microbalance (CAHN Instruments, Inc., Cerritos, California, USA). Aluminum capsules were oven dried at 130°C for 1 h and cooled in a desiccator for 30 min. The capsules were then weighed (M1). Seeds were put in each capsule and oven dried at 103°C for 17 h with the capsules open. The capsules were sealed immediately after drying and cooled in a

desiccator for 30 min. The sealed capsules were weighed again (M2), and individual seed mass of each sample was calculated as (M2 – M1)/number of seeds.

Table 3.4 Seed collections in 2007, 2008, and 2009 from inside and outside the Prairie Heating and CO_2 Enrichment experiment plots, located in Cheyenne, Wyoming.

Species	Collection		
	2007	2008	2009
Bouteloua gracilis	Inside, outside	Inside	
Pascopyrum smithii	Inside, outside		Inside
Koeleria macrantha		Inside, outside	Inside
Hesperostipa comata	Inside	Outside	Inside
Chenopodium leptophyllum		Inside, outside	
Salsola iberica		Inside, outside	Inside
Grindelia squarrosa			Inside
Centaurea diffusa			Inside
Linaria dalmatica		Outside	Inside

3.3.2 Seed viability

For *Bouteloua gracilis*, *Koeleria macrantha*, *P. smithii* and *Hesperostipa comata*, Tetrazolium testing was conducted at the end of the germination test to determine seed viability of the remaining, un-germinated seeds. Seeds were stained with 0.1% Tetrazolium Chloride solution for 24 h at room temperature and then checked under the microscope. *Hesperostipa comata* seeds were first pierced and seeds of *P. smithii* were dissected before soaking. Seeds were assumed viable when embryos were stained evenly red (Grabe, 1970). For *Chenopodium leptophyllum*, *Salsola iberica*, *Gridelia squarrosa*, *Centaurea diffusa*, and *Linaria dalmatica*, seeds with firm embryos when pressed were considered viable at the end of the germination test. The numbers of viable and non-viable seeds were recorded. Seed viability was determined by calculating the portion of viable seeds (germinated and non-germinated) in total filled seeds (viable and non-viable) under each temperature regime for germination test. The maximum

viability under the temperature regimes used for germination was selected as the viability for this species.

3.3.3 Seed fill

For *P. smithii*, *Hesperostipa comata*, *Chenopodium leptophyllum*, *Salsola iberica*, *Gridelia squarrosa*, *Centaurea diffusa*, and *Linaria dalmatica*, filled seeds can be easily separated from empty ones by pressing with fingers. However, for species such as *Bouteloua gracilis* and *Koeleria macrantha*, seeds are light and small, and it is difficult to separate empty seeds from filled ones. For these species, a seed blower was used to separate empty and filled seeds. The heavier seeds falling onto the bottom were further checked for filling using a light table that provided back lighting. Light can transmit through the palea and lemma, but cannot transmit through the solid seeds. If there was an oval or round solid dark part in the middle of the seeds, it was considered as a filled seed. Otherwise, it was considered as an empty seed. Although back lighting improve the accuracy of separating empty and filled seeds, a viability test, as described above, further improved the determination of filled seeds.

The total number of viable and dead seeds was considered as filled seeds in each plot. Seed fill percentage was then determined using the portion of filled seeds in the total seeds under each temperature regime. Mean seed fill percentage was applied to each plot and species.

3.3.4 Seed germination test

Germination tests were conducted using SANYO growth chambers (Sanyo Versatile Environment Chamber MLR-350H, Sanyo Scientific, USA). One of seven alternating temperatures with a temperature amplitude of 10°C was randomly assigned to each chamber (10/0, 12.5/2.5, 15/5, 20/10, 25/15, 30/20, 35/25^{o}C). A preliminary experiment using seeds from outside of the experimental plots was conducted under 24-h darkness, using six temperature

regimes for each species (10/0, 15/5, 20/10, 25/15, 30/20, 35/25°C), with a randomized-complete-block-design (RCBD) with five replicates. Replicates were put into growth chambers at 7 d intervals. Depending on seed availability, 25–100 seeds per Petri dish were used per replicate.

Three or four temperature regimes were chosen for the seeds of each species from inside the PHACE plots, depending on seed availability as well as thermal time model constructing requirements (Table 3.5). Germination tests were conducted under 24-h darkness for all species except *Gridelia squarrosa* with 12/12 h light/darkness. A RCBD with three or five replicates (with the exception of *Chenopodium leptophyllum* which had no replicates and *Salsola iberica* which had two replicates) was used and replicates were put into growth chambers at 2 to 7 d intervals. The number of seeds for each replicate ranged from 10 to 110.

Table 3.5 Alternating temperature regimes with temperature amplitude of 10°C for germination tests of seeds from inside the Prairie Heating and CO_2 Enrichment experiment plots, located in Cheyenne, Wyoming.

Species	Alternating Temperature (°C)						
	10/0	12.5/2.5	15/5	20/10	25/15	30/20	35/25
Bouteloua gracilis	×		×		×		×
Koeleria macrantha	×		×	×	×		
Hesperostipa comata			×	×	×		
Chenopodium leptophyllum	×			×		×	
Salsola iberica	×			×		×	
Gridelia squarrosa			×	×	×	×	
Centaurea diffusa			×		×	×	
Linaria dalmatica	×	×	×	×			

Seeds were imbibed on top of a double layer of filter paper (VWR 413) in 9 cm plastic Petri dishes by adding 4 mL of distilled water. Petri dishes were randomly placed in growth chambers using the middle shelves to minimize temperature differences within chambers. Petri dishes were sealed in clear plastic bags to reduce water evaporation and distilled water was

added when necessary to keep the filter paper moist. Seeds were sprayed with 0.05% benomyl solution whenever growth of microorganisms was observed.

A data logger (21X Campbell Scientific Inc., USA) was set up to continuously monitor temperatures inside each growth chamber. Two temperature probes were placed on the middle shelves of each chamber. Temperatures were recorded at hourly intervals. Measured temperatures were used for thermal time model construction.

Germinated seeds were counted and removed at 1 day intervals. Seeds were considered germinated when the radicle was $\geq$ 2 mm. Germinated seeds were removed after each counting. Germination tests were terminated when no seeds germinated for 14 consecutive days.

3.4 Thermal time model construction

3.4.1 Estimating germination rates of sub-populations using Chapman-Richards growth function

A germination time course curve was constructed for each germination temperature regime and each species using the Chapman-Richards growth function (Richards, 1959; Lee, 2002). The Chapman-Richards growth function takes the following form:

$$g = a(1-\exp(-bt))^c \qquad \text{(Equation 3.1)}$$

where g = germination percentage, t = time, a = the asymptote, b = the rate parameter, and c = the shape parameter. The three parameters, a, b, and c, are constant. A sigmoid growth form has an asymptote (parameter a) for the maximum germination percentage. The b and c parameters together define the shape of the curve.

For modeling purposes, a seed population is considered to be composed of sub-populations based on relative germination rate (Garcia-Huidobro *et al.*, 1982b). A nonlinear

regression procedure with the Levenberg-Marquardt algorithm method was used to estimate parameters (a, b, and c) for the Chapman-Richards growth function and germination time (t_g) for the 10, 20, 30, 40, 50, 60, 70, 80, and 90% sub-populations wherever available. Multiple starting values for the parameters were provided to ensure that the nonlinear least square solution was a global minimum rather than a local minimum (Schabenberger & Pierce, 2002). The germination time for each subpopulation was calculated based on the Chapman-Richards function and used to calculate the germination rate for the construction of a thermal time model. Since there is no real coefficient of determination (R^2) for nonlinear models, model fit was measured using Pseudo-R^2 as follows:

$$\text{Pseudo-}R^2 = 1 - SS(\text{Residual})/SS(\text{Total}_{\text{Corrected}}) \qquad \text{(Equation 3.2)}$$

where Pseudo-R^2 = the approximate value used to measure model fit, SS (Residual) = the sum of the squares of the residual, and SS ($\text{Total}_{\text{Corrected}}$) = the sum of the squares of the corrected total.

3.4.2 Estimating base temperatured and thermal time requirements

Germination rate for sub-population g ($GR_{(g)}$) was calculated with the reciprocal of germination time ($1/t_g$). The base temperature (T_b) and thermal time of each species was estimated using extrapolation (graphical) methods (Garcia-Huidobro *et al.*, 1982a). Temperature was treated as the dependent variable and plotted on $GR_{(g)}$. The intercept of the linear regression line was T_b and the linear relationship between temperature T and $GR_{(g)}$ varies among sub-population in the slope of the regression line, which is equal to the thermal time ($\theta_{(g)}$):

$$T = T_b + \theta_{(g)}GR_{(g)} = T_b + \theta_{(g)}(t_{(g)})^{-1} \qquad \text{(Equation 3.3)}$$

To estimate the common T_b, a linear regression model of T on $GR_{(g)}$ for the available sub-populations with a successive increment of 10% was established. The actual recorded temperatures of the growth chambers were averaged over the time required for the germination of the sub-population and used for thermal time modeling. Sub-optimal temperatures under which the germination rate begins to decrease compared with the previous temperature, were removed when calculating T_b (Wang *et al.*, 2004). Those data points were not following the linear relationship between $GR_{(g)}$ and temperature. Thermal time model was constructed when at least 30% subpopulation germination were reached, with each subpopulation contains at least three temperature points. A sum of square reduction test (Schabenberger & Pierce, 2002) was used to assess if there was a common T_b among the available subpopulation. The full model was composed of different T_bs and $\theta_{(g)}$s for the available subpopulations. The reduced model was composed of one common T_b and different slopes for the subpopulations. The following equation was used for the sum of square reduction test:

F = {(SS(Residual)Reduced - SS(Residual)Full)/(df(Residual)Reduced - df(Residual)Full)}/MSError(Full) (Equation 3.4)

If the result of this test showed that the full and the reduced models were not significantly different ($P \leq 0.05$), a common T_b was used for all subpopulations and the slopes of the subpopulations were based on the common T_b were $\theta_{(g)}$s.

Base temperature (T_b) and thermal time requirement for 50% subpopulation germination (θ_{50}) were calculated for each replicate of each treatment and species. A one-way ANOVA was conducted to determine the treatment effect. Treatment means were separated using least a significant difference (LSD) test with a significance level of $P = 0.05$.

3.5 Data analysis

In addition to the modeling techniques described above, an Analysis of Variance (ANOVA) was conducted using the GLM procedure for individual seed mass, seed viability, and seed fill to determine treatment effects of elevated CO_2 concentrations, heating, and deep and shallow irrigation. Treatment means were separated using least a significant difference (LSD) test at $P = 0.05$.

Because the temperature regimes used for germination and the field treatments may affect the total germination, a two-way ANOVA was conducted to analyze the temperature and treatment effects as well as their interaction with a significance level of $P = 0.05$.

Chapter 4 RESULTS

4.1 Variation in seed germination response to temperature among species

Bielefeld participated the project as a summer student, helping with the lab work of the preliminary experiment. Das has been cited and discussed from her undergraduate thesis. Preliminary germination tests for seeds collected outside of the PHACE plots revealed no statistical differences in germination across temperature regimes in *Bouteloua gracilis* (2007) and *Chenopodium leptophyllum* (2008) (P = 0.80 and 0.38, respectively) (Table 4.1) (Bielefeld, 2009). *Salsola iberica* (2008) and *Bouteloua gracilis* (2007) had high total germination ranging from 80% to 99%, and 75% to 84%, respectively. Total germination of *Hesperostipa comata* (2007), *P. smithii* (2008) and *Linaria dalmatica* (2008) showed a large variation across temperature regimes, indicating species-specific responses to temperature. Higher temperatures (20/10°C, 25/15°C, and 30/20°C) favored the germination of *Hesperostipa comata* (2007) and *P. smithii* (2008), while *Linaria dalmatica* (2008) germination was greatest at the intermediate to lower temperatures (15/5°C and 20/10°C). *Hesperostipa comata* (2007) and *P. smithii* (2008) had the highest total germination of 65% at 20/10°C and of 74% at 25/15°C. Warmer temperatures (25/15°C, 30/20°C) significantly germination of *Linaria dalmatica* (2008) to less than 5%. *Koeleria macrantha* (2008) had the lowest total germination, with less than 40% under all temperature regimes.

Predicted base temperatures for germination (T_b) and thermal time requirements for 50% subpopulation germination (θ_{50}) varied among species (Table 4.1) (Bielefeld, 2009). The θ_{50} was

largest for *P. smithii* (2008), which was >160°C*day, and smallest for *Bouteloua gracilis* (2007), being only about 56°C*day. *P. smithii* (2008) also possessed the highest T_b (5.4°C) among all the species. *Bouteloua gracilis* (2007) had a high T_b (5.0°C) and small θ_{50} (56°C*day), indicating less time was required for complete germination. On the contrary, *Koeleria macrantha* (2008) had a low T_b (0.3°C) and high θ_{50} (146°C*day), suggesting an earlier initiation of germination with more time needed for complete germination. Data were insufficient to calculate θ_{50} for *Linaria dalmatica* (2008). However, *Linaria dalmatica* (2008) also had a low T_b (1.1°C) (Table 4.1) (Bielefeld, 2009).

Table 4.1 Mean total germination (%, mean ± SE), base temperature (T_b), and thermal time requirement for 50% subpopulation germination (θ_{50}) of *Bouteloua gracilis* (2007), *Koeleria macrantha* (2008), *Pascopyrum smithii* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Salsola iberica* (2008), and *Linaria dalmatica* (2008), harvested from outside of the PHACE plots, located in Cheyenne, Wyoming (Bielefeld, 2009).

Species/Year	Final germination percentage (%)						P	T_b	θ_{50}
	Temperature (°C)								
	10/0	15/5	20/10	25/15	30/20	35/25		(°C)	(°C*day)
Bouteloua gracilis (2007)	75.1 ± 5.8a[1]	83.5 ± 3.3a	79.7 ± 5.2a	82.8 ± 3.2a	79.2 ± 4.0a	82.3 ± 5.5a	0.805	5.0	56
Koeleria macrantha (2008)	22.2 ± 1.5ab	16.6 ± 3.9b	36.1 ± 3.1a	25.2 ± 5.0ab	18.9 ± 3.8b	0	0.011	0.3	146
Pascopyrum smithii (2008)	25.0 ± 3.1b	37.1 ± 4.1b	64.8 ± 4.8a	57.5 ± 3.7a	54.1 ± 3.9a	N/A[2]	<0.001	5.4	164
Hesperostipa comata (2007)	32.4 ± 6.2b	22.3 ± 3.4b	67.9 ± 4.6a	73.6 ± 3.0a	67.2 ± 4.9a	N/A[2]	<0.001	4.0	131
Chenopodium leptophyllum (2008)	39.0 ± 5.3a	32.5 ± 3.1a	31.8 ± 4.0a	40.3 ± 4.6a	41.7 ± 2.5a	33.4 ± 4.6a	0.383	4.7	89
Salsola iberica (2008)	98.0 ± 0.9ab	98.0 ± 0.9ab	85.5 ± 2.5bc	80.7 ± 3.9c	86.6 ± 5.7abc	98.9 ± 0.6a	<0.001	3.7	108
Linaria dalmatica (2008)	11.4 ± 1.8c	60.0 ± 3.4a	31.9 ± 2.3b	4.8 ± 1.2cd	1.8 ± 0.5d	N/A[3]	<0.001	1.1	N/A[4]

[1] Means with the same letters within a species were not significantly different ($P \leq 0.05$).
[2] There was not enough seeds to conduct germination test under this temperature regime.
[3] There was no germination under this temperature regime.
[4] Was unable to establish thermal time model for this species.

4.2 Seed fill, viability, and individual seed mass as affected by heating, CO_2 enrichment and irrigation

Seed fill for most of the species studied was above 80%, except for *Bouteloua gracilis* (2007 and 2008) and *Koeleria macrantha* (2008) (Table 4.2). Many seeds of *Bouteloua gracilis* (83%) and in *Koeleria macrantha* (90%) were empty. However, the difference in seed fill was mainly between species rather than among treatments. The only significant treatment effect was found in *Gridelia squarrosa* (2009), where elevated CO_2 concentrations reduced seed fill from about 93% to 71%. There was no significant treatment effect on seed fill for all the other species.

A similar trend was observed in seed viability among species as that in seed fill. Seed viability of *Bouteloua gracilis* (2007 and 2008) and *Koeleria macrantha* (2008) was 52% and 59. For other species studied, seed viability was above 90% (Figure 4.1). Treatments influenced seed viability in *Bouteloua gracilis* (2008) ($P = 0.024$) and *Koeleria macrantha* (2008) ($P = 0.009$), as well as in *Gridelia squarrosa* (2009) ($P = 0.017$). Although there were no replications in *Chenopodium leptophyllum* (2008), seed viability was about 100% (Figure 4.1).

For *Bouteloua gracilis* seeds from heating treatments always possessed the highest viability (70%), while irrigation tended to reduce the seed viability in 2008 ($P = 0.024$) but not in 2007 ($P = 0.48$) (Figure 4.1). Seed viability under CO_2 enrichment (Ct) and the control was not significantly different in 2007 and 2008. CO_2 enrichment alone did not affect seed viability of *Bouteloua gracilis* in 2007 and 2008.

For *Koeleria macrantha* (2008) seeds from heating treatments (cT and CT) had higher viability (79% and 75%, respectively), while irrigation (ct-d and ct-s) and CO_2 enrichment (Ct) had lower viability (40%, 52%, and 52% for ct-d, ct-s, and Ct, respectively), when compared with the control (ct) (56%) (Figure 4.1). Heating alone increased seed viability of *Koeleria*

macrantha (2008) (P = 0.009). Irrigation treatments (ct-d and ct-s) reduced seed viability when compared with either heating alone (cT) or the combination of heating and CO_2 enrichment (CT) (P = 0.009).

For *Gridelia squarrosa* (2009), the highest seed viability (about 100%) was in the control (ct) (Figure 4.1). Deep irrigation (ct-d) and CO_2 enrichment (Ct) reduced seed viability to about 84% and 88%, respectively (P = 0.017). Seed viability under the combination of CO_2 enrichment and heating (CT) was significantly greater than that under deep irrigation (ct-d) (P = 0.017).

Table 4.2 Seed fill (%, mean ± SE) for *Bouteloua gracilis* (2007 & 2008), *Koeleria macrantha* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Grindelia squarrosa* (2009), *Salsola iberica* (2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009) from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

Species/Year	Treatment						Mean ± SE	*P*
	ct-d	ct-s	ct	cT	Ct	CT		
Bouteloua gracilis (2007)	14.8 ± 2.6	16.3 ± 5.8	19.4 ± 2.7	13.5 ± 4.2	27.1 ± 5.8	19.9 ± 5.0	18.5 ± 1.9	0.374
Bouteloua gracilis (2008)	12.5 ± 2.2	17.3 ± 1.8	15.8 ± 4.2	19.7 ± 3.3	17.8 ± 2.2	15.7 ± 3.0	16.5 ± 1.1	0.623
Koeleria macrantha (2008)	13.1 ± 0.9	11.1 ± 0.9	11.7 ± 0.7	8.8 ± 1.4	10.7 ± 0.6	9.4 ± 0.8	10.8 ± 0.5	0.062
Hesperostipa comata (2007)	86.5 ± 3.1	84.1 ± 0.3	79.4 ± 2.5	85.1 ± 5.3	89.5 ± 3.4	83.2 ± 2.3	84.6 ± 1.3	0.406
Chenopodium leptophyllum (2008)	100.0	N/A[1]	99.2	90.8	99.3	97.9	97.5 ± 1.7	N/A
Salsola iberica (2008)	100.0 ± 0.0	N/A[1]	99.8 ± 0.2	99.8 ± 0.2	100.0	99.8 ± 0.2	99.9 ± 0.1	0.803
Grindelia squarrosa (2009)	70.8	N/A[1]	92.7 ± 1.5a[2]	N/A[1]	70.8 ± 5.6b	85.4	80.9 ± 4.3	0.020[3]
Centaurea diffusa (2009)	80.7 ± 9.0	N/A[1]	86.7 ± 8.3	N/A[1]	89.7 ± 1.2	82.0 ± 8.7	84.8 ± 3.4	0.820
Linaria dalmatica (2009)	90.1 ± 4.1	90.4 ± 3.7	97.5	86.9	92.9 ± 1.3	85.3 ± 10.8	90.0 ± 2.4	0.842

[1] There was no seed collected under this treatment in that year.

[2] Means with the same letters within a species were not significantly different ($P \leq 0.05$).

[3] ANOVA procedures were conducted under treatments of ct and Ct, since there were not enough replications under the treatments of ct-d and CT.

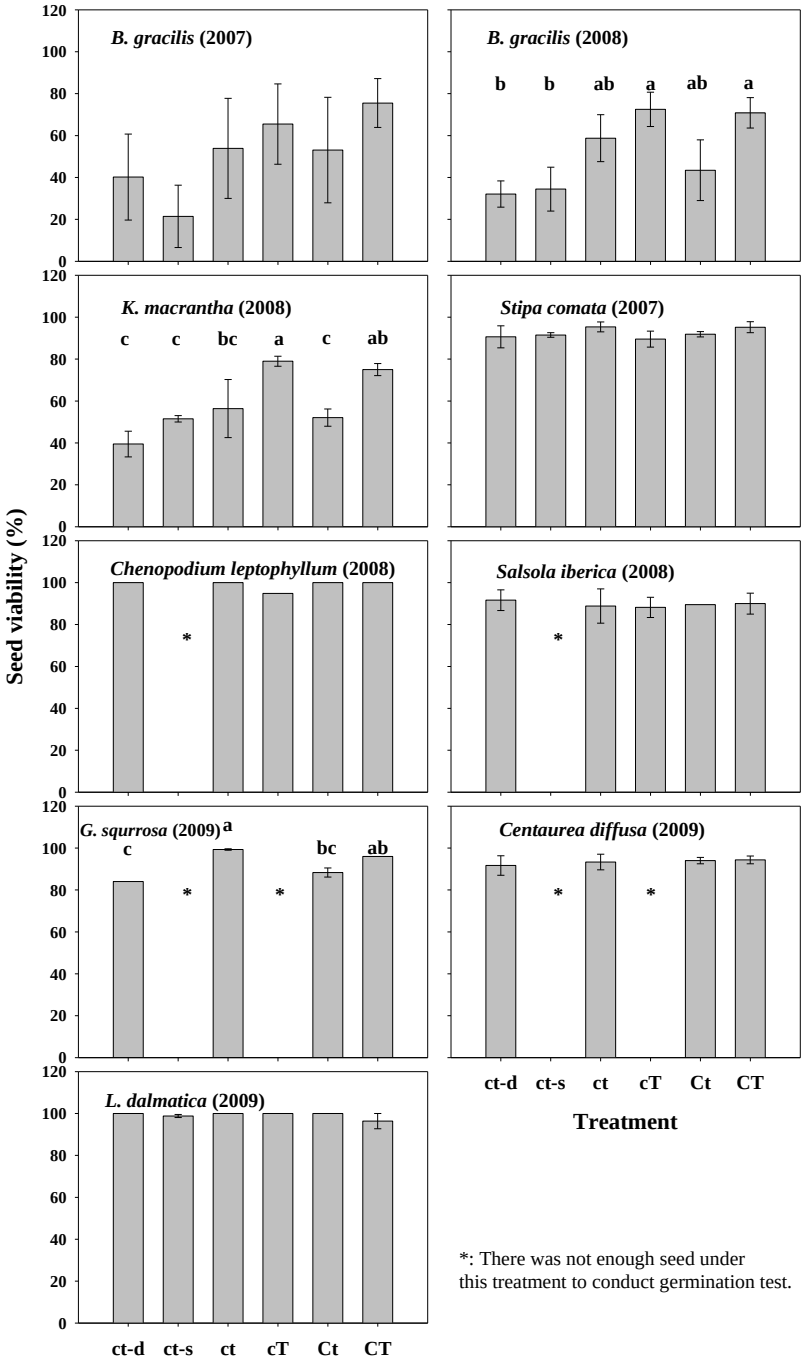

Figure 4.1 Seed viability (%, mean ± SE) for *Bouteloua gracilis* (2007 & 2008), *Koeleria macrantha* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Grindelia squarrosa* (2009), *Salsola iberica* (2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009) from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

Individual seed mass varied among species, ranging from 0.1 mg in *Koeleria macrantha* (2008) and *Linaria dalmatica* (2009), to 4.6 mg in *Hesperostipa comata* (2007) (Table 4.3). However, there was no significant difference in individual seed mass between treatments within species except for *Gridelia squarrosa* (2009) ($P = 0.020$) and *Hesperostipa comata* (2007) ($P = 0.047$). The combination of CO_2 enrichment and heating (CT) increased the individual seed mass of *Gridelia squarrosa* (2009) from 0.42 mg to 1.04 mg, which was also higher than those under the treatments of CO_2 enrichment (Ct) and deep irrigation (ct-d). All treatments tended to increase the individual seed mass of *Hesperostipa comata* (2007), but only heating caused a significant increase in seed mass ($P = 0.047$) (Table 4.3).

Table 4.3 Individual seed mass (mg, mean ± SE) for *Bouteloua gracilis* (2007 & 2008), *Koeleria macrantha* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Grindelia squarrosa* (2009), *Salsola iberica* (2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009) from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

Species/Year	Treatment						Mean ± SE	*P*
	ct-d	ct-s	ct	cT	Ct	CT		
Bouteloua gracilis (2007)	0.32 ± 0.05	0.32 ± 0.02	0.27 ± 0.02	0.36 ± 0.02	0.30 ± 0.04	0.35 ± 0.03	0.32 ± 0.01	0.388
Bouteloua gracilis (2008)	0.28 ± 0.01	0.36 ± 0.05	0.33 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.38 ± 0.09	0.34 ± 0.02	0.731
Koeleria macrantha (2008)	0.124 ± 0.008	0.106 ± 0.005	0.102 ± 0.009	0.094 ± 0.007	0.126 ± 0.010	0.102 ± 0.006	0.109 ± 0.004	0.064
Hesperostipa comata (2007)	4.74 ± 0.14ab[1]	4.57 ± 0.05ab	4.08 ± 0.13b	4.98 ± 0.32a	4.54 ± 0.14ab	4.43 ± 0.10ab	4.56 ± 0.09	0.047
Chenopodium leptophyllum (2008)	0.227 ± 0.012	N/A[2]	0.273 ± 0.030	0.178 ± 0.010	0.204 ± 0.019	0.218 ± 0.003	0.22 ± 0.012	0.078
Salsola iberica (2008)	1.41 ± 0.09	N/A[2]	1.33 ± 0.01	1.46 ± 0.08	1.34 ± 0.23	1.46 ± 0.06	1.41 ± 0.04	0.830
Grindelia squarrosa (2009)	0.67 ± 0.09b	N/A[2]	0.42 ± 0.03b	N/A[2]	0.63 ± 0.03b	1.04 ± 0.12a	0.69 ± 0.09	0.020
Centaurea diffusa (2009)	0.98 ± 0.12	N/A[2]	1.15 ± 0.07	N/A[2]	0.97 ± 0.05	0.82 ± 0.16	0.98 ± 0.06	0.266
Linaria dalmatica (2009)	0.096 ± 0.003	0.089 ± 0.012	0.113	0.112	0.096 ± 0.007	0.085 ± 0.005	0.094 ± 0.004	0.665

[1] Means with the same letters within a species were not significantly different ($P \leq 0.05$)
[2] There was no seed collected under this treatment in that year.
[2] Means with the same letters within a species were not significantly different ($P \leq 0.05$).

4.3 Contrasting the effects of heating, CO_2 enrichment and irrigation on germination of native and invasive species

A wide range of temperature regimes were used for the germination tests. However, high variation in total germination were observed among species, ranging from less than 10% in *Chenopodium leptophyllum* (2008) and *Koeleria macrantha* (2008) to over 80% in *Centaurea diffusa* (2008) (Table 4.4). *Linaria dalmatica* (2009) and *Centaurea diffusa* (2009) had the maximum germination among species, averaging 74% and 82%, respectively.

High temperatures enhanced and low temperatures reduced germination of *Hesperostipa comata* (2007), *Gridelia squarrosa* (2009), *Linaria dalmatica* (2009) and *Salsola iberica* (2008) (Table 4.4). Germination of *Bouteloua gracilis* (2007 and 2008), *Koeleria macrantha* (2008) and *Centaurea diffusa* (2009) was not affected by temperature regime (P = 0.953, 0.199, 0.452 and 0.932, respectively) (Table 4.4, Table 4.5).

Significant treatment effects on total germination were only observed in *Bouteloua gracilis* (2007 and 2008) and *Gridelia squarrosa* (2009) (Table 4.5). In 2007, neither CO_2 enrichment (Ct) nor heating (cT) alone affected total germination of *Bouteloua gracilis*, but their combination (CT) significantly increased germination from 29% to 68% (P = 0.001) (Table 4.4). In 2008, CO_2 alone decreased total germination of *Bouteloua gracilis* from 40% to 26% (ct vs Ct), but increased that from 40% to 59% when combined with heating (ct vs CT) (P < 0.0001). Irrigation treatments reduced germination among all treatments. In 2008, deep (ct-d) and shallow (ct-s) irrigation reduced germination of *Bouteloua gracilis* from 40% to 16% and 40% to 18%, respectively (P < 0.0001). For *Gridelia squarrosa* (2009), deep irrigation decreased total germination from 79% to 51% (P < 0.0001). Elevated CO_2 concentrations alone reduced total germination from 79% to 48% (ct vs Ct), while heating alone increased germination from 48% to

67% when compared with CO_2 enrichment (Ct vs CT). The combination of elevated CO_2 concentrations and heating had no significant effect on total germination (ct vs CT) (Table 4.4).

Total germination for *Koeleria macrantha* (2008) was mostly below 10% and there were no significant treatment effects ($P = 0.853$) (Table 4.5). *Salsola iberica* (2008) had relative total germination, averaging >40% under all treatments. For *Hesperostipa comata* (2007), all treatments tended to increase germination, while for *Chenopodium leptophyllum* (2008), all treatments tended to reduce germination (Table 4.4). *Centaurea diffusa* (2009) had the highest total germination among all the species studied, but it was not affected by treatments ($P = 0.168$) or by temperatures ($P = 0.932$). For *Linaria dalmatica* (2009), germination was high, and either elevated CO_2 concentrations alone (Ct) or combined with heating (CT) tended to reduce germination (Table 4.4).

Table 4.4 Total germination (%, mean ± SE) for *Bouteloua gracilis* (2007 & 2008), *Koeleria macrantha* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Grindelia squarrosa* (2009), *Salsola iberica* (2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009), harvested from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

Species/Year	Temperature	Treatment						Mean ± SE
	(°C)	ct-d	ct-s	ct	cT	Ct	CT	
Bouteloua	10 / 0	35 ± 21	6 ± 6	27 ± 14	50 ± 12	54 ± 29	61 ± 13	39 ± 8A
gracilis	15 / 5	39 ± 21	10 ± 6	38 ± 27	56 ± 28	48 ± 19	72 ± 10	44 ± 8A
(2007)	25 / 15	28 ± 15	17 ± 17	21 ± 15	47 ± 16	50 ± 28	70 ± 13	39 ± 8A
	35 / 25	31 ± 25	1 ± 1	28 ± 8	42 ± 21	60 ± 26	71 ± 15	39 ± 8A
	Mean ± SE	33 ± 9bc	8 ± 4c	29 ± 8bc	49 ± 9ab	53 ± 11ab	68 ± 6a	

Bouteloua	10 / 0	18 ± 5	29 ± 11	49 ± 11	35 ± 6	38 ± 13	62 ± 8	38 ± 4A
gracilis	15 / 5	16 ± 4	11 ± 4	43 ± 8	34 ± 12	16 ± 7	56 ± 9	30 ± 4A
(2008)	25 / 15	18 ± 5	19 ± 6	38 ± 9	47 ± 12	24 ± 11	62 ± 7	35 ± 4A
	35 / 25	14 ± 6	11 ± 4	31 ± 10	33 ± 9	26 ± 12	57 ± 10	29 ± 4A
	Mean ± SE	16 ± 2d	18 ± 3d	40 ± 5b	37 ± 5bc	26 ± 5cd	59 ± 4a	
Koeleria	10 / 0	5.9 ± 0.8	9.4 ± 5.8	11.1 ± 7.3	8.9 ± 4.5	4.2 ± 4.2	10.0 ± 10.0	8.3 ± 2.2A
macrantha	15 / 5	13.6 ± 7.0	11.2 ± 8.2	8.3 ± 4.8	11.9 ± 6.5	12.0 ± 7.2	3.9 ± 3.9	10.2 ± 2.3A
(2008)	20 / 10	4.7 ± 2.4	6.2 ± 4.0	1.7 ± 1.7	11.1 ± 11.1	8.3 ± 6.0	0.0 ± 0.0	5.3 ± 2.1A
	25 / 15	7.3 ± 4.9	4.2 ± 4.2	2.0 ± 2.0	8.9 ± 4.8	6.7 ± 4.1	6.7 ± 6.7	6.0 ± 1.7A
	Mean ± SE	7.9 ± 2.2a	7.7 ± 2.6a	5.8 ± 2.3a	10.2 ± 3.1a	7.8 ± 2.5a	5.1 ± 2.9a	
Hesperostipa	15 / 5	9.4 ± 2.0	7.7 ± 1.8	2.1 ± 2.1	21.0 ± 4.4	17.7 ± 7.4	11.7 ± 1.9	12 ± 2B
comata	20 / 10	30.2 ± 16.2	19.8 ± 2.7	21.8 ± 3.2	24.3 ± 2.5	22.5 ± 12.5	36.0 ± 7.2	26 ± 3A
(2007)	25 / 15	26.1 ± 17.8	24.4 ± 17.5	6.7 ± 3.6	16.7 ± 9.6	26.6 ± 11.4	42.8 ± 4.9	24 ± 5A
	Mean ± SE	22 ± 8a	17 ± 6a	10 ± 3a	21 ± 3a	22 ± 5a	30 ± 5a	
Chenopodium	10 / 0	2.4	N / A*	12.0	1.6	0	0	
leptophyllum	20 / 10	0	N / A*	11.1	0.5	4.0	0	
(2008)	30 / 20	0	N / A*	18.5	0	2.0	0	
Salsola	10 / 0	29 ± 10	N / A*	55 ± 6	65 ± 10	36	59 ± 7	50 ± 7B
iberica	20 / 10	26 ± 3	N / A*	48 ± 1	36 ± 6	51	43 ± 1	40 ± 4B
(2008)	30 / 20	69 ± 8	N / A*	85 ± 7	78 ± 9	77	80 ± 1	78 ± 4A
	Mean ± SE	41 ± 10a	N / A*	63 ± 8a	60 ± 10a	55 ± 12a	60 ± 7a	
Grindelia	15 / 5	43	N / A*	36 ± 13	N / A*	33 ± 8	51	37 ± 6B
squarrosa	20 / 10	59	N / A*	82 ± 5	N / A*	51 ± 7	79	67 ± 8A
(2009)	25 / 15	48	N / A*	96 ± 1	N / A*	61 ± 8	79	75 ± 5A
	30 / 20	76	N / A*	91 ± 4	N / A*	47 ± 6	67	70 ± 6A
	35 / 25	28	N / A*	89 ± 6	N / A*	47 ± 6	59	62 ± 11A
	Mean ± SE	51 ± 6b	N / A*	79 ± 9a	N / A*	48 ± 5b	67 ± 4a	
Centaurea	15 / 5	70.4 ± 13.8	N / A*	85.5 ± 0.4	N / A*	86.6 ± 6.6	89.0 ± 6.8	82.9 ± 3.9A
diffusa	25 / 15	77.1 ± 6.2	N / A*	79.2 ± 11.4	N / A*	84.7 ± 6.8	81.8 ± 4.6	80.7 ± 4.7A
(2009)	30 / 20	69.7 ± 0.3	N / A*	82.8 ± 15.5	N / A*	86.5 ± 10.4	90.2 ± 1.2	82.3 ± 3.7A
	Mean ± SE	72.4 ± 6.1a	N / A*	82.5 ± 5.3a	N / A*	85.9 ± 2.4a	87.0 ± 2.4a	
Linaria	10 / 0	53 ± 10	39 ± 14	50	53	41 ± 13	63 ± 7	50 ± 5B
dalmatica	12.5 / 2.5	86 ± 5	75 ± 10	91	91	75 ± 11	78 ± 17	80 ± 4A
(2009)	15 / 5	95 ± 3	81 ± 10	95	98	88 ± 5	86 ± 7	89 ± 3A
	20 / 10	84 ± 6	65 ± 14	91	80	78 ± 4	73 ± 12	77 ± 4A
	Mean ± SE	80 ± 5a	65 ± 7a	82 ± 11a	81 ± 10a	71 ± 7a	75 ± 6a	

*: There was no seed collected under this treatment in that year. Therefore there was no germination test conducted for this treatment.

Table 4.5 Two-way ANOVA table (*P* value) for total germination of *Bouteloua gracilis* (2007 & 2008), *Koeleria macrantha* (2008), *Hesperostipa comata* (2007), *Chenopodium leptophyllum* (2008), *Grindelia squarrosa* (2009), *Salsola iberica* (2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009), harvested from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation; ambient CO_2, control temperature, shallow irrigation; ambient CO_2, control temperature; ambient CO_2, heating; elevated CO_2 concentrations, control temperature; and elevated CO_2 concentrations, heating.

Species / Year	Temperature	Treatment	Temperature × Treatment
Bouteloua gracilis (2007)	0.953	0.001	1.000
Bouteloua gracilis (2008)	0.199	< 0.0001	0.988
Koeleria macrantha (2008)	0.452	0.853	0.993
Hesperostipa comata (2007)	0.020	0.190	0.794
Salsola iberica (2008)	0.001	0.201	0.857
Grindelia squarrosa (2009)	0.001	<0.0001	0.094
Centaurea diffusa (2009)	0.932	0.168	0.973
Linaria dalmatica (2009)	0.000	0.309	0.998

4.4 Germination thresholds as affected by heating, CO_2 enrichment, and irrigation

Base temperatures for germination (T_b) or thermal time requirements for 50% subpopulation germination (θ_{50}) were not calculated for species with low germination, including *Koeleria macrantha* (2008) (< 20%), *Hesperostipa comata* (2007) (< 30%), and *Chenopodium leptophyllum* (2008) (<20%). For *Salsola iberica* (2008), germination at 10/0°C was low and this temperature seemed to sit outside the sub-optimal temperature range. The remaining two temperatures were not sufficient for thermal time modeling, but the likely range of T_b should be between 10 and 15°C (Figure 4.2). For *Bouteloua gracilis* (2007 & 2008), germination rate under the highest temperature regime (30/20°C) was removed when calculating T_b. There was a sharp increase in germination rate under this temperature regime, which no longer followed the linear relationship (Figure 4.3). A higher T_b would have been calculated if those points were included. T_b and θ_{50} were calculated without those points for *Bouteloua gracilis* (2007 and 2008).

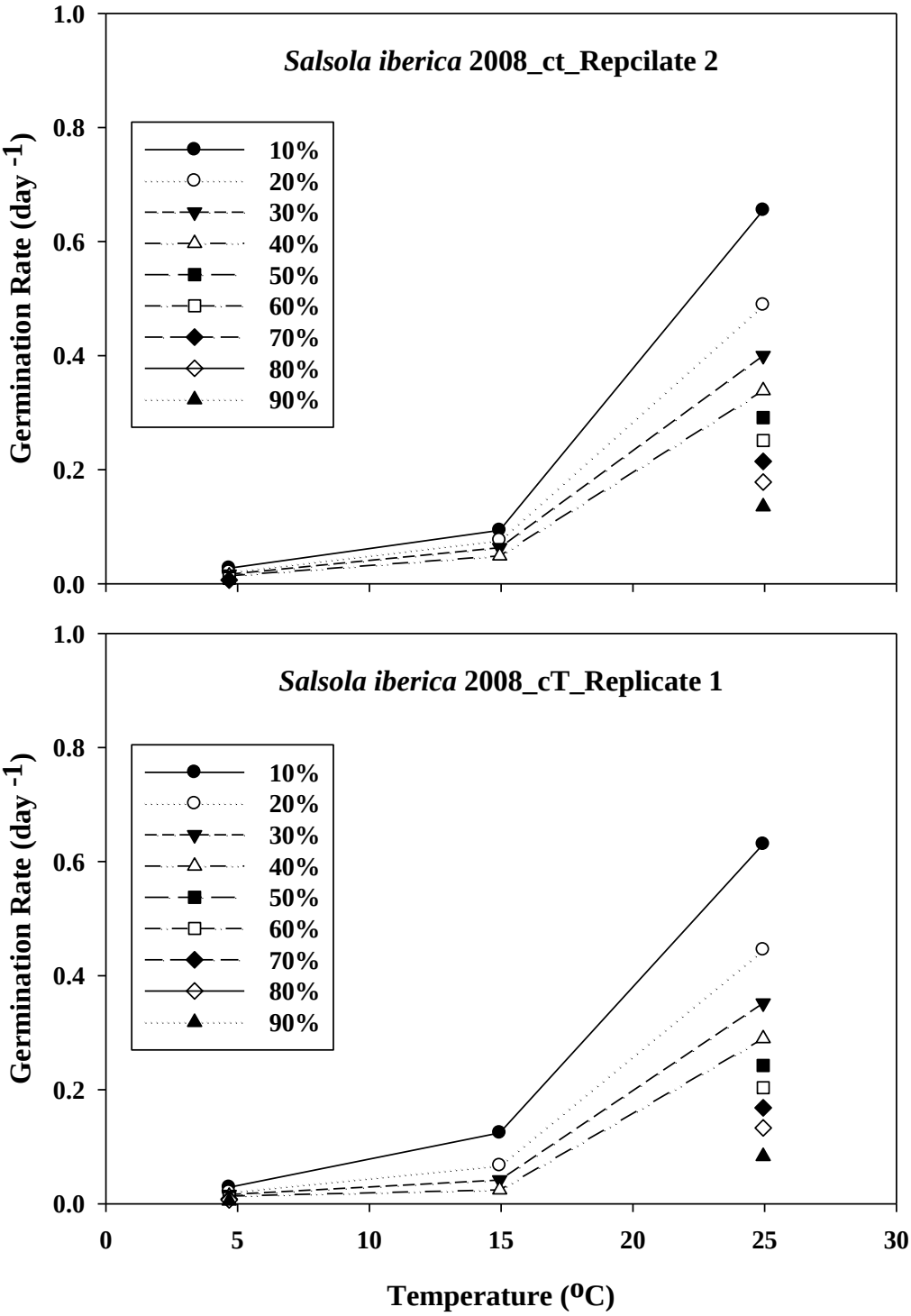
Salsola iberica 2008_ct_Repcilate 2
Germination Rate (day -1)
1.0
0.8
0.6
0.4
0.2
0.0
10%
20%
30%
40%
50%
60%
70%
80%
90%
Salsola iberica 2008_cT_Replicate 1
Germination Rate (day -1)
0
5
10
15
20
25
30
Temperature (oC)

Figure 4.2 Germination rate for 10% - 90% subpopulation of *Salsola iberica* (2008) – replicate 2 of treatment ct (upper graph), and replicate 1 of treatment cT (lower graph).

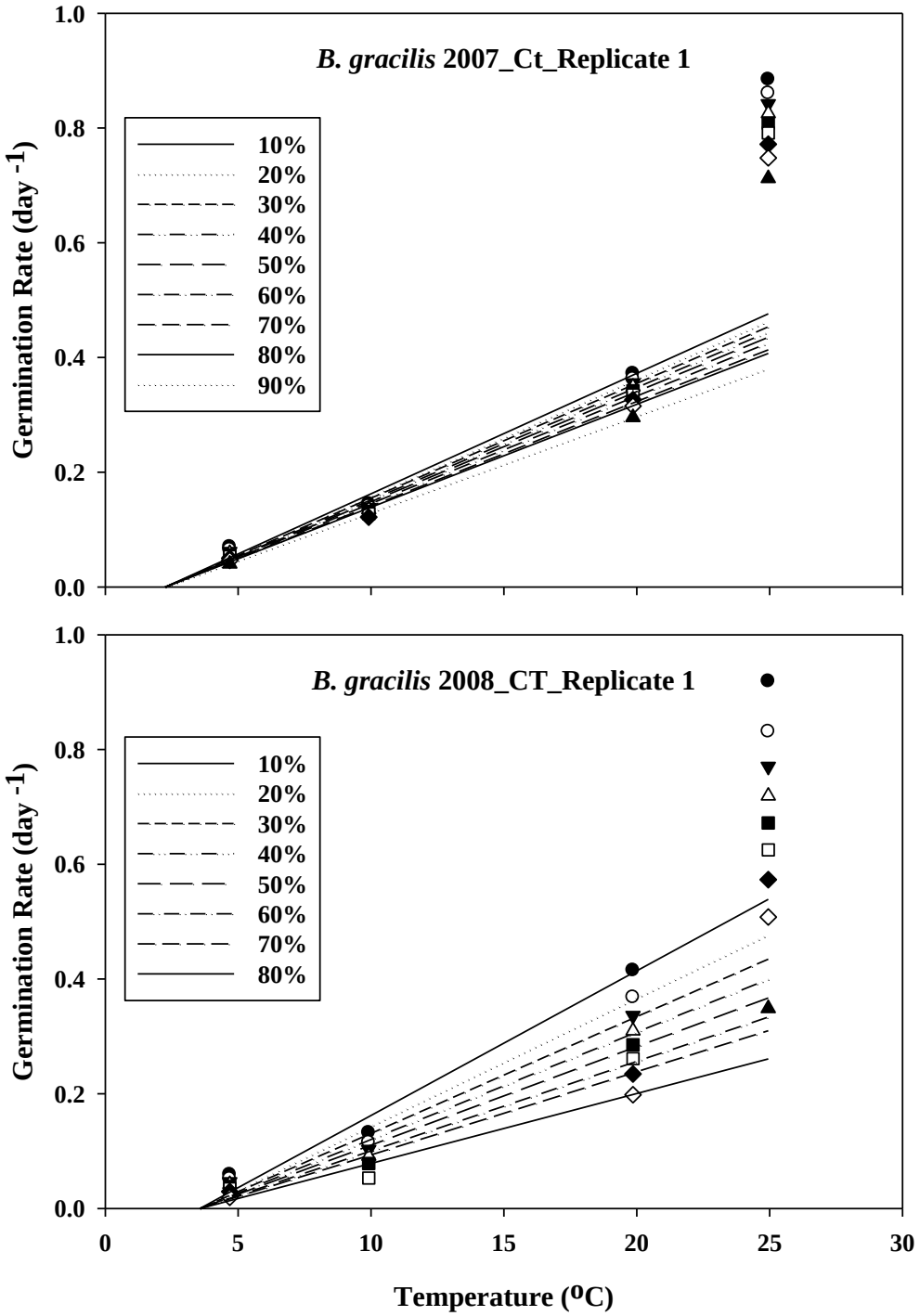

Figure 4.3 Thermal time model for *Bouteloua gracilis* (2007) – replicate 1 of treatment Ct (upper graph), and *Bouteloua gracilis* (2008) – replicate 1 of treatment CT (lower graph).

Thermal time models were constructed for four species; field treatment effects on germination were not significant (Table 4.6). For *Linaria dalmatica* (2009), treatments tended to decrease T_b while increasing θ_{50}, except heating (cT). T_b varied among species. The lowest T_b belonged to *Linaria dalmatica* (2009), averaging -0.6°C (Figure 4.4). The T_b of *Centaurea diffusa* (2009) was 4.6°C, placing it the highest among the three species. *Bouteloua gracilis* had overall T_b of 2.2°C and 2.8°C in 2007 and 2008, respectively. *Centaurea diffusa* (2009) had the smallest θ_{50} among all species, being only 31°C*day (Figure 4.5). The combination of the highest T_b and the lowest θ_{50} enabled *Centaurea diffusa* to finish germination quickly, though it may start germination slowly. On the contrary, *Linaria dalmatica* (2009) had the highest θ_{50}, averaging 105°C*day. Initiation of germination may occur early in the spring when temperatures are low. Similar to the trend in T_b, θ_{50} of *Bouteloua gracilis* was intermediate among species, with θ_{50} in 2008 higher than that in 2007 (85°C*day vs 64°C*day) (Figure 4.5).

Table 4.6 One-way ANOVA table (*P* value) for the base temperature (T_b, °C) and thermal time requirement for 50% subpopulation germination (θ_{50}, °C*day) for *Bouteloua gracilis* (2007 & 2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009).

Species/Year	T_b	θ_{50}
Bouteloua gracilis (2007)	0.981	0.130
Bouteloua gracilis (2008)	0.756	0.828
Centaurea diffusa (2009)	0.967	0.820
Linaria dalmatica (2009)	0.486	0.595

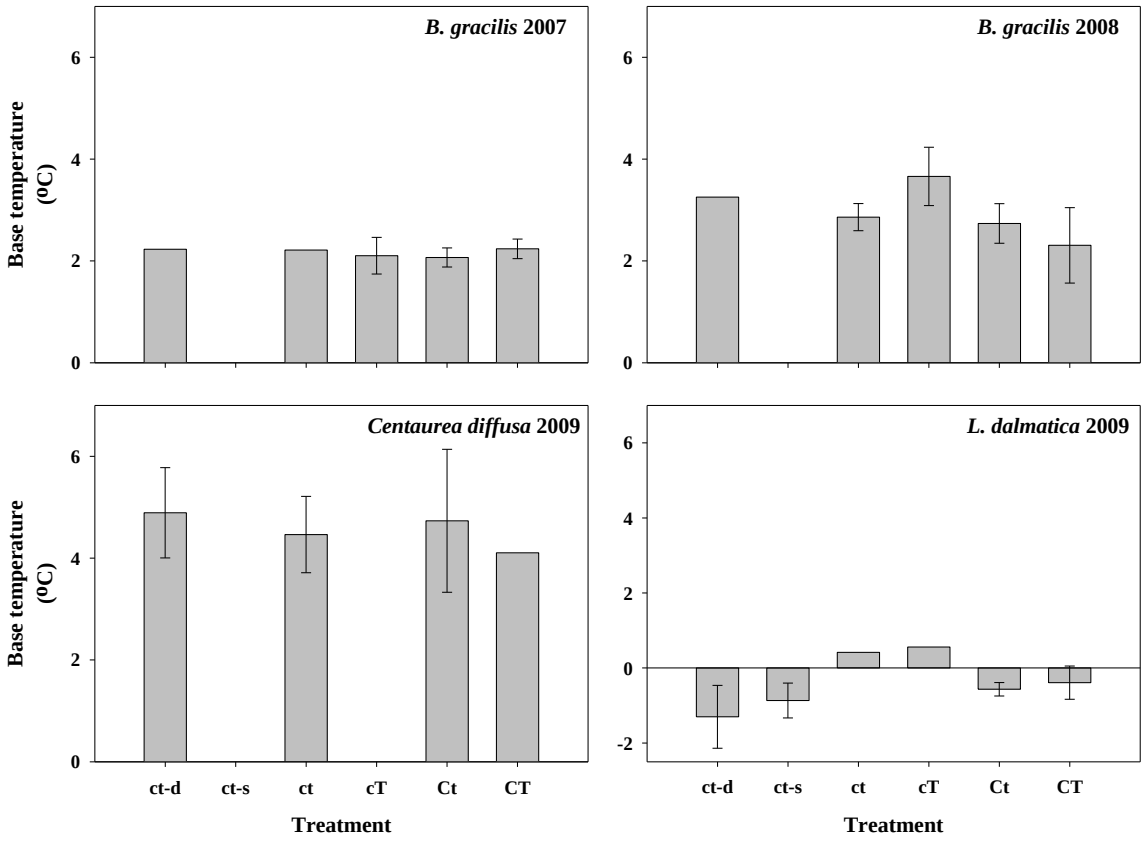

Figure 4.4 Base temperature (T_b, °C) for *Bouteloua gracilis* (2007 & 2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009) from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

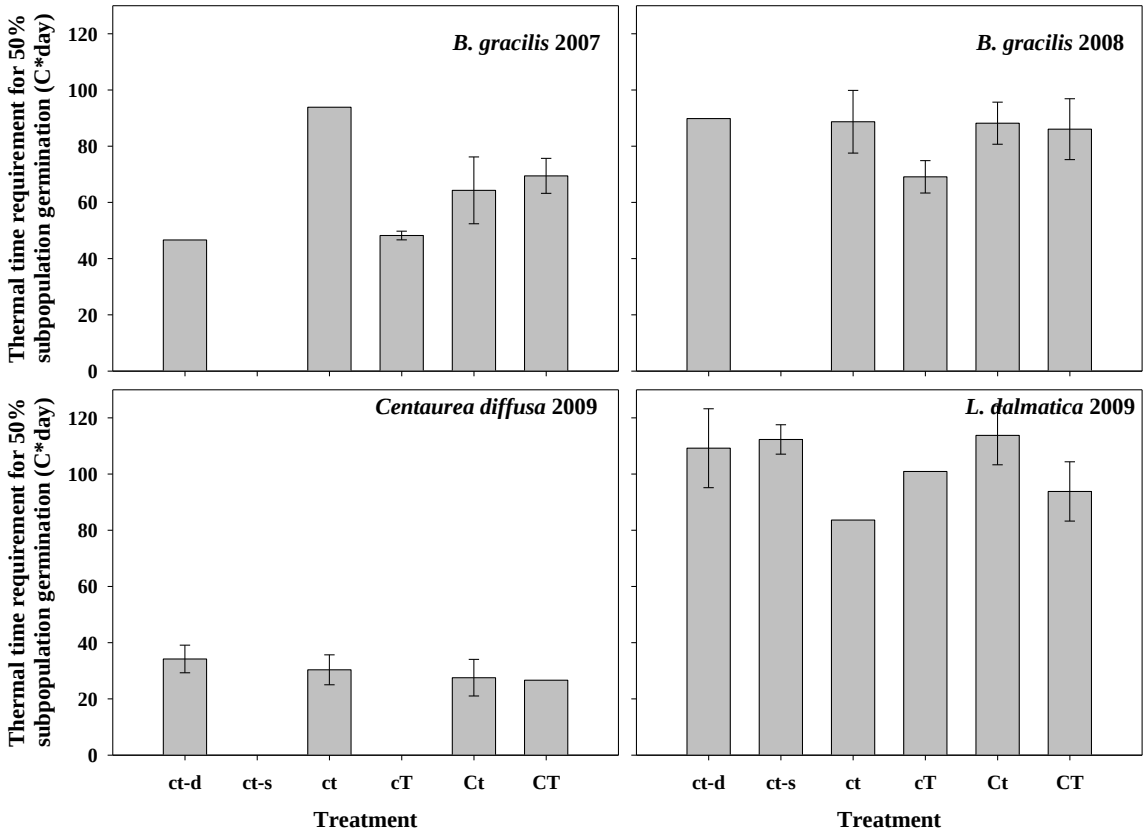

Figure 4.5 Thermal time requirement for 50% subpopulation germination (θ_{50}, °C*day) for *Bouteloua gracilis* (2007 & 2008), *Centaurea diffusa* (2009), and *Linaria dalmatica* (2009) from PHACE plots, located in Cheyenne, Wyoming. Treatments include ambient CO_2, control temperature, deep irrigation (ct-d); ambient CO_2, control temperature, shallow irrigation (ct-s); ambient CO_2, control temperature (ct); ambient CO_2, heating (cT); elevated CO_2 concentrations, control temperature (Ct); and elevated CO_2 concentrations, heating (CT).

Chapter 5 DISCUSSION

5.1 Seed traits as affected by global climate change on the Mixed-grass Prairie

5.1.1 Seed fill, viability and seed mass

Elevated CO_2 concentrations and (or) heating either increased (Jablonski *et al.*, 2002; Thürig *et al.*, 2003; Darbah *et al.*, 2007; Sandvik & Eide, 2009; De Frenne *et al.*, 2010; Gao *et al.*, 2012) or decreased (Kudo *et al.*, 2004; Hovenden *et al.*, 2007; Hovenden *et al.*, 2008; Qiu *et al.*, 2010) seed mass, number of seeds, and seed fill. These changes closely related to seed fill percentage. Among species in the present study, however, the only significant treatment effect was in *Gridelia squarrosa* where CO_2 enrichment reduced seed fill by 22%. There was no apparent trend in seed fill among functional groups, life forms, or between native and invasive species.

Seed viability in *Hesperostipa comata*, *Chenopodium leptophyllum*, *Salsola iberica*, *Centurea diffusa* and *Linaria dalmatica* was high and not affected by treatments intended to simulate global climate change. Seed viability was also high in *Grindelia squarrosa*, but deep irrigation and CO_2 enrichment reduced seed viability. Heating increased seed viability only in *Koeleria macrantha*; CO_2 enrichment reduced seed viability compared with heating, whereas heating + CO_2 enrichment eliminated the negative effect caused by CO_2 enrichment. A trend of reduced seed viability under irrigation and by CO_2 enrichment was also observed in *Bouteloua gracilis*. Reduced seed viability by CO_2 enrichment has been reported in *Arabidopsis thaliana* (Andalo *et al.*, 1996) and *Austrodanthonia caespitosa* (Hovenden *et al.*, 2008). Lower N

concentrations and (or) N content were reported for seeds produced on *A. caespitosa* plants under enriched CO_2 (Hovenden *et al.*, 2008), *Bromus rubens* (Huxman *et al.*, 1998), eight species from a calcareous grassland (Thürig *et al.*, 2003). On the other hand, C concentrations in seeds may (Thürig *et al.*, 2003) or may not (Hovenden et al., 2008) be reduced by elevated CO_2 concentrations. A higher C/N ratio may reduce protein content, therefore leading to decreased seed viability (Andalo *et al.*, 1996; Bai *et al.*, 2003).

Greater seed viability under warming has been reported in alpine or high-latitude ecosystems (Wookey *et al.*, 1995; Kullman, 2002; Lantz *et al.*, 2009). In a semi-arid temperate grassland, heating did not directly affect seed viability in *Leymus chinensis*, but it reduced the proportion of light seeds, which might have low viability (Gao *et al.*, 2012). Warmer environmental conditions may affect the chemical structure of seed and viability through altered allocation of photosynthate to seeds (Vong and Murata, 1997; Wallwork *et al.*, 1998).

Heating or heating + CO_2 enrichment increased individual seed mass in *Hesperostipa comata* and *Gridelia squarrosa*. Seed mass of the other species studied was not significantly affected by treatments. Positive effects of heating on seed mass have been reported in *Beta vulgaris* L. (Wood *et al.*, 1980) and *Glycine max* (L.) Merr. (Seddigh & Jolliff, 1984). Warmer temperatures can increase the rate of seed ripening, but shorten the time for seed filling. Plants grown under warmer environments tended to produce smaller seeds when seeds fill quicker and ripening faster (Fenner, 1992). The 1.5/3.0°C (day/night) rise in temperature in the present study is moderate compared with most heating studies; this moderate increase in temperature may have been not enough to cause an effect on seed mass in most species. Other indirect effects of heating on seed mass include drought (Morgan *et al.*, 2011) and increased soil N availability (Dijkstra *et al.*, 2010). Drought can thicken the seed coat (Hill *et al.*, 1986), producing heavier seeds; or it

can decrease seed mass. However, there is usually a trade-off between seed number or total seed mass and size or individual seed mass (Woodward & Begg, 1976; Izzeldin *et al.*, 1980; Gales & Wilson, 1981). Nutrient addition generally increases seed size or mass by enhancing plant growth during seed filling (Ene & Bean, 1975; Ham *et al.*, 1975; Chakrabarti, 1983; Eck, 1984). Fenner (1992) concluded that nutrient addition can have positive effects on seed size when the amount added increases the nutrient pool above the suboptimal amounts.

Even though CO_2 enrichment did not affect seed mass, greater individual seed mass under elevated CO_2 concentrations was observed in many species, including crops (Krenzer & Moss, 1975; Bai *et al.*, 2003), pasture and old-field species (Newton, 1991), and grasses (Huxman *et al.*, 1998). For 79 crops and wild species, individual seed mass increased an average of 4% under CO_2 enrichment (Jablonski *et al.*, 2002). It should be noted, however, that earlier climate change studies were often conducted in growth chambers or glasshouses (Huxman *et al.*, 1998), usually with only one growing season (Garbutt & Bazzaz, 1984), and often with very high CO_2 concentrations (Bai *et al.*, 2003). General conclusions or hypotheses based on responses observed in controlled environment conditions should be cautious without validation again field experiments (Drake *et al.*, 1985). Furthermore, field conditions are far more complicated than artificially controlled growth conditions (Newton, 1991). Recent climate change studies using Open-top Chambers (OTC) or Free Air CO_2 Enrichment (FACE) systems, enabled field studies under manipulated CO_2 concentrations with relatively stable performance (Newton, 1991), demonstrated slightly different results from the previous studies. Instead of the general enhancing trend, elevated CO_2 concentrations exhibited various as well as species-specific effects on individual seed mass (Thürig *et al.*, 2003; Stiling *et al.*, 2004; Hovenden *et al.*, 2007; Williams *et al.*, 2007). Even though CO_2 enrichment did not affect any species in the

present study, respectively increasing and decreasing trends of seed mass in grasses and forbs were observed.

5.2 Seed germination as affected by global climate change on the Mixed-grass Prairie

Elevated CO_2 concentrations increase the seed germination percentage in *Themeda triandra* and *Leontodon taraxacoides* (Williams *et al.*, 2007), *Scabiosa columbaria* (Thürig *et al.*, 2003), *Plantago lanceolata* (Wulff & Alexander, 1985), and *Acer rubrum* (Mohan *et al.*, 2004) while decreasing seed germination in *Austrodanthonia caespitose*, *Hypochaeris radicata* (Williams *et al.*, 2007), and *Arabidopsis thaliana* (Andalo *et al.*, 1996). Elevated CO_2 concentration had no effect on seed germination of *Bromus rubens* (Huxman *et al.*, 1998), three species on a natural-poor Calcareous grassland in Switzerland (Thürig *et al.*, 2003), six lines of *Triticum aestivum* (Bai *et al.*, 2003), and three scrub-oak species (Stiling *et al.*, 2004). In the present experiments, germination of most species was not affected by CO_2 enrichment. However, elevated CO_2 concentrations significantly reduced germination in *B. gracillis* and *Gridelia squarrosa* in 2008 while it increased germination in *B. gracillis* in 2007. Seeds of *Bouteloua gracilis* (2007 and 2008) and *Gridelia squarrosa* produced under CO_2 enrichment also tended to germinate slower compared with those under ambient CO_2 concentrations. Seeds from plants grown under elevated CO_2 concentrations had reduced seed quality, seed size, and seed germination (Andalo *et al.*, 1996). CO_2 enrichment during plant growth can modify seed filling and maturation processes, and lower seed protein content by increasing the C/N ratio in seeds, reducing seed viability (Andalo *et al.*, 1996). Plants grown under elevated CO_2 concentrations produced more empty seeds with lower viability and greater individual seed mass in *Gridelia squarrosa*. CO_2 enrichment tended to reduce seed viability in *B. gracillis* as well. Decreased seed

quality in *B. gracillis* and *Gridelia squarrosa* caused by elevated atmospheric CO_2 concentrations might have slowed and reduced germination.

Various responses in seed germination caused by warming during plant growth and seed development have been reported; germination was greater in shrubs and forbs in subarctic ecosystems (Graae *et al.*, 2008; Milbau *et al.*, 2009), trees and herbaceous species (Thompson & Naeem, 1996; Chidumayo, 2008; De Frenne *et al.*, 2010), and *Themeda triandra*, a grass (Williams *et al.*, 2007). Warming reduced seed germination in a subarctic, dwarf shrub (Graae *et al.*, 2008), three woody species in Africa (Chidumayo, 2008), and grasses in Eurasian grasslands (Gao *et al.*, 2012) and temperate grasslands of Austrilia (Williams *et al.*, 2007), or no effect in *Leymus chinensis* (Gao *et al.*, 2012) and 20 subarctic species (Milbau *et al.*, 2009). In the present study, germination of seeds from *Bouteloua gracillis* and *Gridelia squarrosa* was greater under warming. Heating increased germination in *Gridelia squarrosa* when compared with elevated CO_2 concentrations (CT vs Ct), and in *B. gracillis* when combined with CO_2 enrichment (CT vs ct). A trend of increased germination of seeds with warming was observed in *Koeleria macrantha*, *Hesperostipa comata*, and *Centaurea diffusa*, while a decreasing trend in seed germination was observed in *Salsola iberica* and *Linaria dalmatica*. A positive correlation between temperature during seed development and germination was demonstrated in *Rosa sp.* (Von Abrams & Hand, 1956), *Chenopodium bonus-henricus* (Dorne, 1981), and *Stellaria media* (Van der Vegte, 1978). Warmer conditions during seed production can reduce seed dormancy (Fenner, 1991). Such effects were reported in *Dactylis glomerata* (Probert *et al.*, 1985), *Plantago lanceolata* (Alexander & Wulff, 1985), *Festuca arundinacea* (Boyce *et al.*, 1976), and *Avena fatua* (Sexsmith, 1969). Exceptions, however, were found in *Chenopodium album* (Karssen, 1970), *Glycine max* (Keigley & Mullen, 1986), and *Stylosanthes hamata* (Argel & Humphreys,

1983). Greater synthesis of germination-promoting substances, such as abscisic acid by higher temperatures (Goldbach & Michael, 1976) and (or) drought (Peters, 1982; Sawhney & Naylor, 1982), or inhibitory substances by lower temperature, such as delaying the onset and reducing the rate of production of α–amylase in wheat and barley (Nicholls, 1980) may be the underlining mechanism. Heating can cause desiccation of seeds, especially in the semi-arid Mixed-grass Prairie (Morgan *et al.*, 2011). Drought during seed filling decreased germination in *Glycine max* (Dornbos *et al.*, 1989) possibly because a thickened seed coat increased dormancy (Hill *et al.*, 1986).

As one of the dominant species in North American prairies (Dijkstra *et al.*, 2010), response of *Bouteloua gracilis* to global climate change can have substantial impact on the structure and function in grasslands. CO_2 enrichment reduced germination in *Bouteloua gracilis*, but the combination of elevated CO_2 concentrations and warming increased the germination. Peak total aboveground biomass of C_4 grasses occurred under the combination of elevated CO_2 concentrations and heating (Morgan *et al.*, 2011). CO_2 enrichment countered the desiccating effect of warming, thus there was no change in soil water content under CT (Morgan *et al.*, 2011). Elevated CO_2 concentrations alone did not benefit *Bouteloua gracilis*, but when it was combined with heating, growth and germination of this native C_4 grass increased (Morgan *et al.*, 2011).

Recruitment of *Bouteloua gracilis* from seeds is rarely observed in natural populations in the Shortgrass Steppe (Hyder *et al.*, 1971; Lauenroth *et al.*, 1994b). Occurrence of seedling recruitment and seedling survival are dependent on the amount of annual precipitation (Laycock, 1989 & 1991), soil texture (Lauenroth *et al.*, 1994b), seed viability (Coffin & Lauenroth, 1990), and competition from adult plants (Aguilera & Lauenroth, 1993). A prevailing view was that

Bouteloua gracilis is not able to regenerate from seeds when annual precipitation is <380mm (Laycock, 1991). However, emergence of seedlings during some dry years and the lack of occurrence during some wet years indicates the importance of the effectiveness of soil water (Lauenroth *et al.*, 1994b). Average annual precipitation on the PHACE site is 388mm (Parton *et al.*, 2007), which was just above the hypothesized threshold for germination. Climate change is predicted to alter the magnitude and seasonality of precipitation, with or without changing the total amount (Christensen *et al.*, 2007). It is also predicted to increase extreme rainfall events (Harper *et al.*, 2005). These changes in precipitation make it difficult to predict seedling recruitment of *Bouteloua gracilis* under future climatic conditions. Although *Bouteloua gracilis* seeds can germinate rapidly in warm, moist soil, the initiation and development of adventitious root system 6-10 weeks after germination is depend upon sufficient water in the soil at a depth of 6 mm or less (Hyder *et al.*, 1971). Parton *et al.* (2007) predicted a decrease of soil water content at the depth of 4-15 cm with heating during the growing season on the PHACE site. Elevated CO_2 concentrations were predicted to increase soil water content at the 4-15 cm depth, but to have no effect during very wet or very dry period (Parton *et al.*, 2007). Heating-induced desiccation might be detrimental for seedling survival of *Bouteloua gracilis*. CO_2-induced water saving cause more water allocation in the deeper layer of soil, benefiting plants with deep, tap roots (Morgan *et al.*, 2007) rather than shallow rooted species like *Bouteloua gracilis*. Soil water content (5-25 cm) was unaffected under the combination of elevated CO_2 concentrations and heating (CT) (Morgan *et al.*, 2011), indicating that the future CT conditions may have little effect on seedling survival of *Bouteloua gracilis*. However, seedling establishment of *Bouteloua gracilis* may be further constrained by intraspecific competition after the development of adventitious root system (Aguilera & Lauenroth, 1993).

There two irrigation treatments were designed to mimic the water saving effects caused by CO_2 enrichment, but their effects on seed traits including germination did not always agree with that of elevated CO_2 concentrations. The irrigation treatments had more negative effects on seed traits of *Bouteloua gracilis* than CO_2 enrichment. The poor performance of *Bouteloua gracilis* under watering treatments may be due to its limited tolerance to wet, poorly drained soils (Stubbendieck *et al.*, 1992).

5.3 Interaction between native and invasive species pairs in the Mixed-grass Prairie as affected by global climate change

Chenopodium leptophyllum and *Salsola iberica* are annual forbs, with the former a native, C_3 species and the later an invasive C_4 species. *Chenopodium leptophyllum* has the tendency to be weedy (Spira & Wagner, 1988), it has a persistent seed bank (Thorne *et al.*, 2007). *Chenopodium leptophyllum* initiates rapid germination of some seeds after exposure to suitable conditions, but some seeds persist in the soil seed bank (Haukos & Smith, 2001). Therefore, *Chenopodium leptophyllum* is capable of surviving and persisting in adverse environment. Rowe *et al.* (2009) observed fewer *Chenopodium leptophyllum* seedlings establishing under reduced N availability. CO_2 enrichment reportedly reduces soil inorganic N (Dijkstra *et al.*, 2010), which may in turn reduce seedling emergence of *Chenopodium leptophyllum*. Heating may increase soil N availability (Dijkstra *et al.*, 2010), but germination of seeds produced in the heating plots in the present study was not significantly affected. On the contrary, heating tended to decrease individual seed mass, viability, and germination in *Chenopodium leptophyllum*. Therefore, *Chenopodium leptophyllum* may not be favored by future climatic conditions. On the other hand, *Salsola iberica* can quickly germinate when conditions for germination are suitable (California Department of Food and Agriculture, 2002) and

complete germination within a short time especially under higher temperatures (Young & Evans, 1972). *Salsola iberica* is also capable of germinating with small amounts of rainfall (Young *et al.*, 1995). On top of those inherent advantages in regeneration, the observed responses suggest climate change treatments tended to increase the individual seed mass and to increase seed viability without changing the seed fill in *Salsola iberica*. Heating treatments also increased plant height in wet and dry years (Blumenthal, personal communication). On the other hand, CO_2 enrichment tended to decrease the germination. Overall, when compared with *Chenopodium leptophyllum*, *Salsola iberica* may be able to better adapt to future climatic conditions regarding regeneration.

Gridelia squarrosa is capable of surviving and growing under adverse conditions (Nuzzo, 1976), but it does not tolerate frequent disturbance (Donaldson & Mazet, 2011). *Centaurea diffusa*, one of the most exotic, invasive weedy species in western North America, is competitive and aggressive (Lacey *et al.*, 1990). Seed traits and germination of these two forbs responded differently to simulated global climate change. Elevated CO_2 concentrations decreased the seed fill, seed viability, and germination in *Gridelia squarrosa*, but seeds tended to be heavier. Heating, on the contrary, tended to have a positive effect on all seed traits and germination. The combination of CO_2 enrichment and heating (CT), which is more represent the future climate conditions (Morgan *et al.*, 2011), increased individual seed mass, but tended to have negative effects on other seed traits and germination in *Gridelia squarrosa* in 2009, a relatively wet year (Morgan *et al.*, 2011). No treatments significantly affected the seed traits and germination in *Centaurea diffusa*. However, clear trends were observed. Plants grown under CO_2 enrichment and warmer conditions, either alone or in combination, tended to produce more empty seeds with lower individual seed mass, but higher viability and germination. Wet years did not influence

treatment effects on seed characteristics and germination in *Centaurea diffusa*. The tumbleweed characteristic could enable it to invade wider area over longer distances (USDA Forest Service, 2006). Overall, *Centaurea diffusa* may have regeneration advantages when compared with *Gridelia squarrosa* under future climatic conditions, especially during wet years. Regeneration of *Gridelia squarrosa* may not change appreciably under future climate conditions.

Native and invasive species displayed different strategies in response to simulated climate change. Native, C_3 forbs generally responded negatively to elevated CO_2 concentrations and positively to heating in seed traits and germination. However the combination of elevate CO_2 concentrations and heating often offset each other, indicating the native, C3 forbs are more resilient to simulated climate change than invasive species. For invasive C_3 forbs, climate change treatments tended to either increase or decrease seed traits, depending on species and trait. For example, all treatments tended to increase seed viability and seed germination while reducing seed mass and seed fill in *Centaurea diffusa*. Invasive species appear to be more opportunistic and are able to respond to environmental changes quickly for survive (Rahel & Olden, 2008; Shine, 2011).

5.4 Potential invasion of Linaria dalmatica to the Mixed-grass Prairie as affected by global climate change

When grown under warmer conditions, *Linaria dalmatica* tended to produce more empty seeds, but seed viability, individual seed mass and seed germination were not significantly changed. Elevated CO_2 concentrations did not benefit the reproduction of *Linaria dalmatica*. With a trend of decreasing individual seed mass, elevated CO_2 concentrations tended to reduce seed germination, although it did not affect the seed fill or seed viability. The combination of CO_2 enrichment and heating (CT), tended to negatively affect all seed traits and germination of

Linaria dalmatica. No climate change treatments significantly affected seed characteristics and germination. However, it is notable *Linaria dalmatica* had the second lowest base temperature required for germination among all the species studied, reflecting its tolerance to low temperatures during germination (Vujnovic & Wein, 1997). Species with a low T_b is able to initiate germination early in the spring.

Elevated CO_2 concentrations increased while heating decreased heights of *Linaria dalmatica* (Blumenthal *et al.*, 2013). Similar treatment effects were observed on plant survival, which was probably due to the water saving effect induced by elevated CO_2 concentrations. On the other hand, with CO_2 enrichment, heating increased seed mass and did not impede flowering in *Linaria dalmatica* (Blumenthal *et al.*, 2013). Overall, *Linaria dalmatica* can recruit more but taller seedlings and plants can produce similar proportions of viable seeds, and tended to produce smaller seeds with relatively low germination under elevated CO_2 concentrations. With higher temperature, seedling establishment may be poor, plants may be shorter, but flowering, seed setting, seed mass and germination may not be adversely affected. The combination of elevated CO_2 concentrations and heating did not impede plant growth or seedling survival (Blumenthal *et al.*, 2013), nor benefited seed traits and germination. Therefore, potential reproduction of *Linaria dalmatica* may be favored by CO_2 enrichment, but it may not be affected by heating.

Chapter 6 Conclusions

The effects of maternal conditions including CO_2 enrichment, heating, and irrigation on seed properties were species specific. Seed fill of most species studied was not affected by elevated CO_2 concentrations or heating, except for *Gridelia squarrosa* in which elevated CO_2 concentrations reduced seed fill. Reduced seed viability under maternal CO_2 enrichment and greater seed viability under heating was also reported for many species and in various ecosystems. In a similar vein, warming increased seed viability in *Koeleria macrantha*, while elevated CO_2 concentrations reduced seed viability in this grass as well as in *Gridelia squarrosa*. Heating, with or without elevated CO_2 concentrations increased the seed mass in *Hesperostipa comata* and *Gridelia squarrosa*. Elevated CO_2 concentrations reduced total germination and germination rate in *Bouteloua gracilis* (2008 collection) and *Gridelia squarrosa* (2009 collection). Heating, on the contrary, increased the total germination in *Bouteloua gracilis* when combined with CO_2 enrichment, and in *Gridelia squarrosa* when compared with elevated CO_2 concentrations. Elevated CO_2 concentrations and heating seemed to have opposite effects on seed production, seed characteristics, and germination. CO_2 enrichment caused more negative effects while heating offered more positive effects on seed characteristics and germination. In the semi-arid Mixed-grass Prairie, positive effects of heating could easily be eliminated by drying brought about by warming, unless the positive effect was caused or strengthened by drought. Therefore, the combination of elevated CO_2 concentrations and heating often mollify one other, causing no significant change in seed traits or germination.

As previously reported, CO_2 enrichment decreased seed viability, total germination, and germination rate. Warming could directly affect seed viability by influencing the chemical structure of seeds and (or) indirectly by plants producing heavier seeds with higher viability. Heating-induced drought and increased soil N availability may have enabled plants to produce seeds with greater mass. Reduced dormancy and (or) greater synthesis of "germination-promoting" substances under warmer conditions could further increase germination.

Many species studied were not significantly affected by field treatments in this study. The 1.5/3.0°C (day/night) warmer temperatures used in this study were relatively low compared with other studies, which might explain the responses observed. In addition, early climate change studies were often conducted with very high CO_2 concentrations, under controlled conditions. Furthermore, inter- and (or) intra-specific competition was not a factor in controlled environment studies. Field validation of laboratory results was often not included.

Overall performance under future climate conditions would appear to be specific to every species. *Bouteloua gracilis* can be expected to grow and produce seeds that can germinate under the predicted climate conditions in the future. *Gridelia squarrosa*, however, showed neutral or negative responses to simulated global climate change. Furthermore, under the predicted, future climate conditions, *Gridelia squarrosa* may be less adapted as compared with *Centaurea diffusa*; *Salsola iberica* could be more adapted as compared with *Chenopodium leptophyllum*. *Linaria dalmatica* might be more competitive under climate change, because elevated CO_2 concentrations increased its seed production. Such species-specific changes in seed quality and germination as affected by climate change could exert substantial cumulative effects on community composition in the long run. The distribution and abundance of *Bouteloua gracilis*

may be favored by global climate change. By contrast, invasion by non-native species such as *Linaria dalmatica* could prosper and be more abundant under future climate conditions.

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